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(54) Title: GENE EXPRESSION MODULATED BY ACTIVATION OF MICROGLIA OR MACROPHAGES

(57) Abstract

Polynucleotides, polypeptides, kits and methods are provided related to regulated genes characteristic of microglia and macrophages.

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Gene Expression Modulated By Activation Of Microglia Or Macrophages

Background of the Invention

Microglia have been implicated as key players in the inflammatory responses associated with numerous degenerative brain pathologies. For example, it has been shown that microglia activation is involved in such degenerative brain conditions as trauma, abscess, focal ischemia, experimental allergic encephalitis (EAE), Wallerian degeneration, Down's syndrome and Alzheimer's disease (Griffen et al., In: Biology and Pathology of Astrocyte-Neuron Interactions, pp. 359-381 (Fedoroff et al., eds., (1993)). Recently, it has also been shown that during HIV infection, activation of the inflammatory response leads to astrogliosis and neuronal loss, pathologies that correlate with progressive AIDS dementia (Merrill et al., FASEB J., 5:2391-2397 (1991)).

Present information related to the characterization of microglial cells in their quiescent and various activated forms is incomplete. One hypothesis is that while the various microglial subtypes may arise from the differentiation of cells from a common precursor pool that is possibly indistinguishable from that giving rise to macrophage and dendritic cells, the roles played by differentiated microglia in normal neural physiology and neuropathology are determined in part by the ensembles of proteins that are expressed after their differentiation. Also, there may be overlapping ensembles expressed during different types of inflammation. In addition to the lack of information regarding quiescent and activated microglia phenotypes, due to the lack of phenotypic markers which distinguish microglial cells from macrophages, it has been difficult to discern the relative contribution of microglia versus infiltrating macrophages during the inflammatory response.

Recent studies indicate that within the central nervous system (CNS), microglia and macrophages are important reservoirs of HIV in infected patients (Gendelman et al., J. Leuk. Biol., 56:389-398 (1994); Perry et al., J. Leuk. Biol., 56:399-406 (1994); Dickson et al., Glia, 7: 75-83 (1993); McGeer et al., Glia, 7: 84-92 (1993)). HIV infection of microglia is thought to lead to their activation and result in the production of factors that initiate a cascade of neuropathological events, leading to a progressive dementia correlated with astrogliosis and neuronal loss (Wiley et al., Ann. Neurol., 29:651-657 (1991); Everall et al., J. Neuropathol. Exp. Neurol. 52:561-566; Lipton, Mol. Neurobiol. 8:181 (1994); Merrill et al., FASEB J., 5:2391-2397 (1991)).

The neurophysiology associated with HIV infection shares similarities with the neurodegenerative features observed in humans and experimental animal models of other neuropathological conditions, such as brain trauma, experimental allergic encephalitis (EAE), Wallerian degeneration after nerve transection, brain abscess, focal ischemia, Down's syndrome and Alzheimer's disease. See Griffin et al., In: Biology and Pathology of Astrocyte-Neuron Interactions, pp. 359-381 (Fedoroff et al., eds. (1993)); Stanley et al., J. Neuropathol. and Exp. Neurol., 53:231-238 (1994); Griffin et al., Neurosci. Lett., 176:133-136 (1994)). Further, many of the neuropathological findings have been observed in brains at autopsy of HIV-seropositive individuals in the absence of opportunistic infections, suggesting that these features are a direct consequence of HIV infection (Stanley et al., 1994).

The inflammatory processes that lead to neurodegeneration are presumably, at least in part, exaggerations of normal interactions between brain microglia, astroglia, oligodendrocytes and neurons. Such interactions may include, for example, those that normally facilitate synaptic plasticity in neurons, as well as those that facilitate myelinogenesis by oligodendrocytes. Evidence suggests that some of the molecules produced by activated microglia contribute to the neurodegeneration associated with HIV infection. For example, studies have shown that injection of interleukin-1 (IL-1), a product of activated microglia, can produce some of the neuropathologies associated with HIV-induced neurodegeneration (Giulian et al., J. Neurosci., 8:2485-2490 (1988)). Similarly, transgenic expression of the HIV envelope glycoprotein gp120 or transgenic expression of IL-6 by astrocytes, has been shown to mimic HIV-induced

neuropathologies (Toggas et al., Nature. 367:188-193 (1994); Campbell et al., Proc. Natl. Acad. Sci., 90:10061-10065 (1993)).

In addition, nitric oxide (NO), produced by nitric oxide synthase (iNOS), an enzyme induced in activated microglia, has been shown to contribute to neuronal degeneration. For example, it has been demonstrated in primary cortical cultures that nitric oxide mediates the neurotoxicity associated with human immunodeficiency virus type-1 coat protein (Dawson et al., Proc. Natl. Acad. Sci., 90:3256-3259 (1993); Wallas et al., Neuroreport, 5:245-248 (1993)). Others have reported both neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds (Lipton et al., Nature, 364:626-632 (1993)). It is likely that additional products of microglial cells, presently unknown, contribute to the pathological process.

The microglia are bone marrow-derived cells of monocyte lineage that, like peripheral macrophages, demonstrate remarkable phenotypic plasticity dependent upon their environment. Dawson et al., (1993); Wallas et al., (1993); Lipton et al., (1993)). While the exact relationship of microglia to macrophages has not been definitively determined, it is known that in addition to NO and IL-1, microglia produce an array of cytokines. In addition, several studies indicate that microglia may also serve as antigen-presenting cells during an inflammatory response (Frei et al., Eur. J. Immunol. 17:1271-1278 (1987); Carson et al., Glia 22:72-85 (1998)). Interestingly, Carson et al. has shown that mature microglia resemble immature antigen-presenting cells (Carson et al., 1998). Further studies demonstrate that CNS microglial cell activation and proliferation follow direct interaction with tissue-infiltrating T-cell blasts (Sedgewick et al., J. Immunol., 160:5320-5330 (1998)).

At least five forms of CNS macrophages have been described based on their morphologies and reactivity with reagents that recognize various macrophage cell surface antigens. These forms include amoeboid, ramified, activated, reactive, and perivascular microglia (Flaris et al., Glia 7:34-40 (1993)). Cumulatively, these forms account for 10-20% of the cells of the CNS, a percentage far greater than the concentration of macrophages found in peripheral tissues, which is fewer than 1% of the cells (Lawson et al., Neurosci., 39:151-170 (1990)).

Most tissues contain resident bone marrow-derived antigen-presenting cells, ranging from classic macrophages, as found in spleen red pulp and lung alveolae, to macrophage-like Kupffer cells resident in liver and pancreas, to dendritic cells found in spleen, skin and lymph nodes. Dendritic cells are highly efficient antigen-presenting cells, while macrophages are 10- to 100-fold less efficient (Steinman, Ann. Rev. Immunol., 9:271-256 (1991); Knight et al., Curr. Opin. Immunol., 5:374-382 (1993); Levin et al., J. Immunol., 151:6742-6750 (1993); Sprent et al., Internatl. Immunol., 1:517-525 (1989)). Conversely, unlike dendritic cells, macrophages are highly phagocytic, and can form multinucleated giant cells in granulomatous inflammatory responses. The type of antigen-presenting cell may influence whether the response of an activated T lymphocyte is primarily a TH1 response (associated with IFNγ and TNFα production and inflammation) or a TH2 response (associated with IL-4, IL-5 and TGFα production) (Stout, Curr. Opin. Immunol., 5:398-403 (1993); Mosmann et al., Ann. Rev. Immunol., 7:145-173 (1989); Janeway et al., Immunol. Rev., 101:39-80 (1988)). Interestingly, some of these cytokines have been implicated both in immune responses and in normal CNS development (Wucherpfennig, Clin. Immunol. Immunopathol., 72:293-306 (1994); Jonakait et al., Neuron, 12:1149-1159 (1994); Merrill, J.E., Dev. Neurosci. 14:1-10 (1992)).

Parenchymal microglia display many of the same expression markers as macrophage and dendritic cells (Carson et al., (1998)). Consequently, in past, they have been presumed to share many of the same functions as these cells. However, recent studies have demonstrated that while microglia do share some functional similarities with these cells, they have a distinctly different repertoire of responses (Carson et al., (1998); Sedgewick et al., (1998)). Two features specific to microglia may represent CNS specializations. First, microglia possess ATP-stimulated inward rectifying potassium channels, whereas peritoneal macrophages have an outward rectifying potassium channel (Kettenmann et al., Glia, 7:93-101 (1993)). Consequently, microglia are especially sensitive to depolarizing events and the release of ATP from injured cells.

Second, in contrast to peripheral macrophages, microglia are weak antigen-presenting cells (Perry et al., J. Leuk. Biol., 56:399-406 (1994); Carson et al., (1998); Sedgewick et al., (1998); Flaris et al., Glia, 7:34-40 (1993)). Unlike peripheral tissue macrophages, microglia in

healthy CNS tissue express costimulatory molecules necessary to activate T lymphocytes during antigen presentation, but do not express the MHC class II necessary to present the antigen. In response to pathology, microglia do express MHC class II, but are very slow to acquire the ability to present antigen. Indeed, some studies have suggested that they may induce T cell apoptosis rather than T cell proliferation (Sedgewick et al., (1998)). Other studies show that microglia acquisition of antigen-presenting function is coupled with their production of soluble factors (prostaglandins) which suppress T lymphocyte proliferation and activation. These soluble factors may also act to suppress the ability of infiltrating macrophage to activate T cells in the CNS. It is thought that these features of microglia may represent CNS specializations that prevent autoimmune attack of nonregenerating neurons under normal conditions, and a controlled response under pathological conditions.

To determine the relationship between microglia and macrophages, Flaris et al. generated a panel of monoclonal antibodies (MAbs) raised against activated rat microglia purified by cell culture procedures (Glia, 7:34-40 (1993)). Presumably, as a result of the methodology used, the MAbs react exclusively with cell surface antigens and secretory proteins, most of which are presently unknown. Flaris et al. showed that the MAbs differentiated the microglia of normal CNS from active microglia, however, none of the limited set of MAbs distinguished between activated microglia and activated macrophages. This finding is consistent with the concept that microglia and macrophages are highly related cell types that adopt a particular phenotype depending upon environmental conditions. Further, the MAbs detected phenotypic markers that were induced on microglia in different patterns under different inflammatory conditions, suggesting that different microglia forms may contribute selectively to the pathophysiologies associated with different inflammatory responses. Indeed, both microglia and macrophages show phenotypic heterogeneity even within a single pathology, illustrating their sensitivity to environmental activators (Perry et al., (1994); Flaris et al., (1993); Williamson et al., J. Neuroimmunol., 32:199-207 (1991)).

Similar to microglia, macrophage effector function is dependent upon the type of activation, and may include an array of partially characterized responses, including the production of cytokines, proteases, and reactive oxygen and/or nitrogen intermediates. For example, LPS stimulation of macrophages results in the production of TNFα, IL-1 and IL-6, but

not nitric oxide. However, studies aimed at identifying the cascade of activation events have concentrated on only a few readily followed molecules (iNOS, IFN) and have yielded conflicting data, in part due to the phenotypic plasticity of macrophages (Levin et al., J. Immunol., 151:6742-6750 (1993)).

Thus, the identification and characterization of molecules that are selectively expressed in subsets of microglia would greatly illuminate the physiology of this system. First, the identification of proteins induced by activation may contribute to the understanding of the neuropathology responsible for dementia and other neurological diseases. Additionally, the proteins induced in active microglia may lead to the identification of neural-specific proteins, which would distinguish microglia functionally from other macrophages. Unfortunately, at present, only a few already-identified species have been candidates for study. As a result of such limited studies, present understanding of the neuropathology associated with neurodegenerative conditions is incomplete.

Accordingly, there remains a need to define patterns of gene expression that would distinguish microglia, i.e. the resident myeloid cell of the CNS, from macrophages that infiltrate the CNS during inflammation. Such a systematic characterization of microglial-specific versus macrophage-specific proteins would allow: 1) the nature of the relationship between microglia and other monocyte-derived cell types to be precisely determined, and 2) the separation of the relative contributions of microglia and macrophage toward neuroprotection versus neurodegeneration. Furthermore, determining patterns of gene expression that distinguish microglia from macrophages would identify molecules that would be useful to distinguish these two cell types in histological sections of CNS pathology.

Additionally, there remains a need to define patterns of gene expression that would distinguish normal or resting microglia from activated microglia. Such patterns could be determined, for example, by identifying the gene expression regulated by the inflammatory response (e.g. LPS/IFNγ stimulation). Such a systematic characterization would allow the identification of harmful molecules that contribute to the neuroinflammatory pathologies associated with neurodegenerative conditions. Identification of potentially harmful gene products is important to identify molecules that could be useful as a diagnostic markers

indicating neuropathology. Additionally, identification of potentially harmful gene products is important to identify molecules that could be amenable to pharmaceutical intervention. A systematic characterization would also allow the identification of beneficial molecules that contribute to conditions of neuroprotection. Such identification of beneficial products could lead to the development of pharmaceutical agents useful in the treatment of neurodegenerative conditions. Furthermore, the identification of harmful and beneficial products may lead to new lines of study towards the amelioration of symptoms associated with neuroinflammatory pathologies.

SUMMARY OF THE INVENTION

The PCR-based Total Gene Expression Analysis (TOGA) differential display system has been used in studies to examine the differential gene expression in microglia and macrophage cells in both the unstimulated and stimulated (activated) states. Specifically, the TOGA system has been used to analyze and compare the expression patterns of thousands of genes in four cellular conditions: (1) unstimulated microglia; (2) activated microglia; (3) unstimulated macrophage; and (4) activated macrophage.

The present invention provides novel polynucleotides and the encoded polypeptides that are useful for detecting and treating neuroinflammatory pathologies. Additionally, the provided polynucleotides and polypeptides are useful for detecting and treating processes mediated by the activation of microglia. The provided polynucleotides and polypeptides also are useful for detecting and treating processes mediated by the activation of macrophages. The provided polynucleotides and polypeptides also are useful for detecting and treating neurodegenerative processes. The provided polynucleotides and polypeptides also are useful for detecting and treating infections of the nervous system.

In general, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting disorders related to the polypeptides, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying binding partners of the polypeptides.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

Figure 1 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases GTTC, showing PCR products produced from mRNA extracted from (A) untreated microglia (control), (B) microglia treated with LPS/IFNγ (100 ng/ml LPS; 100 U/ml IFNγ) for 22 hours, (C) untreated macrophages (control) and (D) macrophages treated with LPS/IFNγ (100 ng/ml LPS; 100 U/ml IFNγ) for 22 hours, where the vertical index line indicates a PCR product of about 426 b.p. that is present in microglia, but not macrophage cells;

Figure 2 is a graphical representation of a more detailed analysis of the 426 b.p. PCR product indicated in Figure 1, using the extended TOGA primer G-A-T-C-G-A-A-T-C-C-G-G-G-T-T-C-A-A-C-C-G-C-G-T-G-A-A-G-G-T (SEQ ID NO: 55);

Figure 3 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases GTTG, showing PCR products produced from of mRNA extracted from (A) untreated microglia (control), (B) microglia treated with LPS/IFNγ (100ng/ml LPS; 100U/ml IFNγ) for 22 hours, (C) untreated macrophages (control) and (D) macrophages treated with LPS/IFNγ (100ng/ml LPS; 100U/ml IFNγ) for 22 hours, where the vertical index line indicates a PCR product of about 244 b.p. that is present in treated microglia, and enriched in, untreated macrophages and treated macrophages;

Figure 4 is a graphical representation of more detailed analysis of the 244 b.p. PCR product indicated in Figure 3, using the extended TOGA primer G-A-T-C-G-A-A-T-C-C-G-G-G-T-T-G-C-A-C-C-T-A-T-T-G-C-A-T-G-T (SEQ ID NO: 54).

Figure 5A-C shows northern blot analyses of clone MM_3 (AAGT 366), where an agarose gel containing 2μg of poly A enriched mRNA from various murine tissue and cells was blotted after electrophoresis and probed with radiolabeled MM_3. Cells from mixed glial cultures, whole brain tissue, peritoneal macrophage cultures, kidney fibroblast cultures, and bone marrow-derived dendritic cell cultures were either untreated (control), treated with LPS (50 ng/ml), or treated with LPS/IFN-γ (50 ng/ml LPS; 10 U/ml IFN-γ) prior to mRNA isolation. Tissues from lung, heart, kidney, liver, spleen, lymph node, testis, and several brain regions, including the cortex, midbrain, brainstem, and cerebellum, were untreated (5C).

Figure 6 shows northern blot analyses of clone MM_11 (AGGT 315), where an agarose gel containing 10μg of total cytoplasmic RNA from various murine cells was blotted after electrophoresis and probed with radiolabeled MM_11. Microglial, macrophage, and dendritic cells were either untreated (control), treated with LPS (50 ng/ml), or treated with LPS/IFN-γ (50 ng/ml LPS; 10 U/ml IFN-γ) prior to RNA isolation.

Figure 7A-B shows northern blot analyses of clone MM_12 (ACCA 381), where an agarose gel containing 10μg of total RNA from microglial, macrophage, and dendritic cells (7A) or 2μg of poly A enriched mRNA from untreated lung, heart, kidney, liver, spleen, lymph node, testis and several brain tissues (7B) was blotted after electrophoresis and probed with radiolabeled MM_12. Cells from mixed glial cultures, peritoneal macrophage cultures, and bone marrow-derived dendritic cultures were either untreated (control), treated with LPS (50 ng/ml), or treated with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFN-γ) for 22 hours prior to RNA isolation.

Figure 8A-B shows northern blot analyses of clone MM_18 (TTGG 262), where an agarose gel containing 2μg of poly A enriched mRNA from microglial and macrophage cells (8A) or 2μg of poly A enriched mRNA from untreated lung, heart, kidney, liver, spleen, lymph node, testis and several brain tissue (7B) was blotted after electrophoresis and probed with radiolabeled MM_18. Microglia cells from mixed glial cultures and peritoneal macrophage cells were either untreated (control) or treated with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFN-γ) for 1 hour or 22 hours prior to RNA isolation.

Figure 9 is a schematic drawing of the known GOLLI molecules and clone MM_18, demonstrating the relationship between GOLLI molecules and MM_18.

Figure 10 shows northern blot analyses of clone MM_20 (TGTG 411), where an agarose gel containing 2μg of poly A enriched mRNA from murine spleen, brain, microglial cells and macrophage cells was blotted after electrophoresis and probed with radiolabeled MM_20. Brain and spleen tissues were prepared from neonatal (postnatal day 1, P1) or adult mice. Microglia cells from mixed glial cultures and peritoneal macrophage cells were either untreated (control) or treated with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFN-γ) for 1 hour or 22 hours prior to RNA isolation.

Figure 11A-B shows northern blot analyses of clone MM_21 (TCAT 410), where an agarose gel containing 2μg of poly A enriched mRNA from murine spleen, brain, microglial cells and macrophage cells (10A) or 2μg of poly A enriched mRNA from untreated lung, heart, kidney, liver, spleen, lymph node, testis and several brain tissues (10B) was blotted after electrophoresis and probed with radiolabeled MM_21. Brain and spleen tissues were prepared from neonatal (postnatal day 1, P1) or adult mice. Microglia cells from mixed glial cultures and peritoneal macrophage cells were either untreated (control) or treated with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFN-γ) for 1 hour or 22 hours prior to RNA isolation.

Figure 12A-C shows northern blot analyses of clone MM_23 (TCGG 314), where an agarose gel containing 2μg of poly A enriched mRNA from murine spleen, brain, microglial cells and macrophage cells (11A,C) or 2μg of poly A enriched mRNA from untreated lung, heart, kidney, liver, spleen, lymph node, testis and several brain tissues (11B) was blotted after electrophoresis and probed with radiolabeled MM_23. Brain and spleen tissues were prepared from neonatal (postnatal day 1, P1) or adult mice. Microglia cells from mixed glial cultures and peritoneal macrophage cells were either untreated (control) or treated with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFN-γ) for 1 hour or 22 hours prior to RNA isolation.

Figure 13 shows comparisons of the predicted amino acid sequence of DDP with predicted polypeptides from an S. *pombe* gene (SPAC 13G6.04), a human EST yv59a08.s1, and the MM_23 sequence.

Figure 14 shows the sequence and cloning sites of MM_23 used to construct the prokaryote PBAD-TOPO expression vector shown in Figure 13.

Figure 15 is a map of the PBAD-TOPO expression vector construct containing the MM_23 translation sequence.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1-25. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the

coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO: 1-25, or the complement thereof, or the cDNA. "Stringent hybridization conditions" refers to an overnight incubation at 42° C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3'

terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well-described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme

moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formulation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS – STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTT'RANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci., 663:48-62 (1992)).

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The translated amino acid sequence, beginning with the methionine, is identified although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

SEQ ID NO: 1-25 and the translations of SEQ ID NO: 1-25 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified

from the translations of SEQ ID NO:1-25 may be used to generate antibodies which bind specifically to the secreted proteins encoded by the cDNA clones identified.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

The present invention also relates to the genes corresponding to SEQ ID NO:1-25, and translations of SEQ ID NO:1-25. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologues. Species homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method

described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

Signal Sequences

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. Therefore, from a deduced amino acid sequence, a signal sequence and mature sequence can be identified.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., Protein Engineering 10:1-6 (1997)), which predicts the cellular location of a protein based on. the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence corresponding to the translations of SEQ ID NO:1-25 which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

"Identity" per se has an art-recognized meaning and can be calculated using published techniques. (See, e.g.: COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, (1988); BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, (1993); COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, (1994); SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, (1987); and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, (1991)). While there exists a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, et al., SIAM J. Applied Math., 48:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in "Guide to Huge Computers," Martin J. Bishop, ed., Academic Press, San Diego, (1994), and Carillo, et al., (1988)). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux, J.. et al., Nuc. Acids Res., 12:387 (1984)); BLASTP, BLASTN, FASTA (Atschul, et al., J. Molec. Biol. 215:403 (1990)); and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) (using the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981)).

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, 95% identical to a reference sequence, the parameters are set so that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

A preferred method for determining the best overall match between a query sequence (a

sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). The term "sequence" includes nucleotide and amino acid sequences. In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB search of a DNA sequence to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=l, Joining Penalty=30, Randomization Group Length=0, and Cutoff Score=l, Gap Penalty=5, Gap Size Penalty 0.05, and Window Size=500 or query sequence length in nucleotide bases, whichever is shorter. Preferred parameters employed to calculate percent identity and similarity of an amino acid alignment are: Matrix=PAM 150, k-tuple=2, Mismatch Penalty= 1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=l, Gap Penalty=5, Gap Size Penalty=0.05, and Window Size=500 or query sequence length in amino acid residues, whichever is shorter.

As an illustration, a polynucleotide having a nucleotide sequence of at least 95% "identity" to a sequence contained in SEQ ID NO:1-25 means that the polynucleotide is identical to a sequence contained in SEQ ID NO:1-25 or the cDNA except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the total length (not just within a given 100 nucleotide stretch). In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to SEQ ID NO:1-25, up to 5% of the nucleotides in the sequence contained in SEQ ID NO:1-25 or the cDNA can be deleted, inserted, or substituted with other nucleotides. These changes may occur anywhere throughout the polynucleotide.

Further embodiments of the present invention include polynucleotides having at least 80% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to a sequence contained in SEQ ID NO:1-25. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the polynucleotides having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity will encode a polypeptide identical to an amino acid sequence contained the translations of SEQ ID NO:1-25.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference polypeptide, is intended that the amino acid sequence of the polypeptide is identical to the reference polypeptide except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the total length of the reference polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Further embodiments of the present invention include polypeptides having at least 80% identity, more preferably at least 85% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence contained in translations of SEQ ID NO: 1-25. Preferably, the above polypeptides should exhibit at least one biological activity of the protein.

In a preferred embodiment, polypeptides of the present invention include polypeptides having at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98%, or 99% similarity to an amino acid sequence contained in translations of SEQ ID NO:1-25.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem., 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli et al., J. Biotech., 7:199-216 (1988)).

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem., 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-l a. They used random mutagenesis to generate over 3,500 individual IL- l a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract). In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus. the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science, 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham et al., Science, 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted

amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (Pinckard et al., Clin. Exp. Immunol., 2:331-340 (1967); Robbins et al., Diabetes, 36: 838-845 (1987); Cleland et al., Crit. Rev. Therap. Drug Carrier Systems, 10:307-377 (1993)).

Polynucleotide and Polypeptide Fragments

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in that shown in SEQ ID NO:1-25. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in that shown in SEQ ID NO:1-25. These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, and more nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, to the end of SEQ ID NO:1-25. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in the translations of SEQ ID NO:1-25. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most

preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, or 61 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50 or 60, amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions. substrate binding region, and high antigenic index regions. Polypeptide fragments of the translations of SEQ ID NO:1-25 falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the

polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response (See, for instance, Geysen et al., Proc. Natl. Acad. Sci., 81:3998- 4002 (1983)).

Fragments which function as epitopes may be produced by any conventional means (see, e.g., Houghten, R. A., Proc. Natl. Acad. Sci., 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope (see, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe, J. G. et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (see, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985)). A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)).

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A 0232 262). Alternatively, deleting the Fc part after the fusion protein has been

expressed. detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (see, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)).

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. *coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters

will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, PNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXTl and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite

chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1-25. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:1-25 will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: A Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease (disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Assuming one megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. The polynucleotides of SEQ ID NO:1-25 can be used for the analysis of individuals.

First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all

affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (i.e. triple helix formation, see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1360 (1991)) or to the mRNA itself (i.e. antisense sequence, Okano, J. Neurochem. 56:560 (1991)); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from

the current limitations of "Dog Tags" which can be lost, switched. or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals (Erlich, H., PCR Technology, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a southern blot probed with DNA corresponding to the DQa class H HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or

other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen et al., J. Cell. Biol., 101:976-985 (1985); Jalkanen et al., J. Cell . Biol., 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled

Antibodies and Their Fragments," Chapter 13, in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Biological Activities

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases

associated with the biological activity. Thus. the polynucleotides and polypeptides could be used to treat the associated disease.

Nervous System Activitiy

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of neuroblasts, stem cells or glial cells. A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system, by activating or inhibiting the mechanisms of synaptic transmission, synthesis, metabolism and inactivation of neural transmitters, neuromodulators and trophic factors, expression and incorporation of enzymes, structural proteins, membrane channels and receptors in neurons and glial cells.

The etiology of these deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular nervous system disease or disorder. The disorder or disease can be any of Alzheimer's disease, Pick's disease, Binswanger's disease, other senile dementia, Parkinson's disease, parkinsonism, obsessive compulsive disorders, epilepsy, encephalopathy, ischemia, alcohol addiction, drug addiction, schizophrenia, amyotrophic lateral sclerosis, multiple sclerosis, depression, and bipolar manic-depressive disorder. Alternatively, the polypeptide or polynucleotide of the present invention can be used to study circadian variation, aging, or long-term potentiation, the latter affecting the hippocampus. Additionally, particularly with reference to mRNA species occurring in particular structures within the central nervous system, the polypeptide or polynucleotide of the present invention can be used to study brain regions that are known to be involved in complex behaviors, such as learning and memory, emotion, drug addiction, glutamate neurotoxicity, feeding behavior, olfaction, viral infection, vision, and movement disorders.

Immune Activitiy

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation,

differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotide or polypeptide of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polypeptide or polynucleotide of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polypeptide or polynucleotide of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotide or polypeptide of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response,

particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoinunune disorders.

Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polypeptide or polynucleotide of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotide or polypeptide of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polypeptide or polynucleotide of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

A polypeptide or polynucleotide can be used to treat or detect hyperproliferative disorders, including neoplasms. A polypeptide or polynucleotide of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polypeptide or polynucleotide of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Infectious Disease

A polypeptide or polynucleotide of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, the polypeptide or polynucleotide of the

present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox , hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning,

Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using a polypeptide or polynucleotide of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (see, Science 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage,

tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide of the present invention.

Chemotaxis

A polynucleotide or polypeptide of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, a polynucleotide or polypeptide of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (see, Coligan et al., Current Protocols in Immunology 1(2), Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product

mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the invention, (b) assaying a biological activity, and (c) determining if a biological activity of the polypeptide has been altered.

Other Activities

A polypeptide or polynucleotide of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide or polynucleotide of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide or polynucleotide of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide or polynucleotide of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities. Preferably, a polypeptide or polynucleotide of the present invention may be used to change a mammal's mental state or physical state by influencing the response to acute exposure to opiates and opioids, tolerance to opiates and opioids and withdrawal from opiates and opioids.

A polypeptide or polynucleotide of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 80% , preferably at least 85%, more preferably at least 90%, most preferably at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:1-25.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:1-25 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the clone sequence and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:1-25 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the start codon and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NO:1-25.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:1-25 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the first amino acid of the signal peptide and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NO:1-25.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:1-25.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:1-25.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:1-25 beginning with the nucleotide at about the position of the 5' nucleotide of the first amino acid of the signal peptide and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NO:1-25.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:1-25.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:1-25, which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said

sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:1-25.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 3, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of. a nucleotide sequence of SEQ ID NO:1-25.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:1-25. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in an amino acid sequence translated from SEQ ID NO:1-25.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in acids in an amino acid sequence translated from SEQ ID NO:1-25, in the range of positions beginning with the residue at about the position of the first amino acid of the secreted portion and ending with the residue at about the last amino acid of the open reading frame.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in an amino acid sequence translated from SEQ ID NO:1-25.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in an amino acid sequence translated from SEQ ID NO:1-25.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to acids in an amino acid sequence translated from SEQ ID NO:1-25.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25, which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25..

Also preferred is the above method wherein said step of comparing sequences is

performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encodes a polypeptide comprising an amino acid sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of amino acid sequences translated from SEQ ID NO:1-25. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The present invention also includes a diagnostic system, preferably in kit form, for assaying for the presence of the polypeptide of the present invention in a body sample, such brain tissue, cell suspensions or tissue sections, or body fluid samples such as CSF, blood, plasma or serum, where it is desirable to detect the presence, and preferably the amount, of the polypeptide of this invention in the sample according to the diagnostic methods described herein.

In a related embodiment, a nucleic acid molecule can be used as a probe (an oligonucleotide) to detect the presence of a polynucleotide of the present invention, or a gene corresponding to a polynucleotide of the present invention, or a mRNA in a cell that is diagnostic for the presence or expression of a polypeptide of the present invention in the cell. The nucleic acid molecule probes can be of a variety of lengths from at least about 10, suitably about 10 to about 5000 nucleotides long, although they will typically be about 20 to 500 nucleotides in length. Hybridization methods are extremely well known in the art and will not be described further here.

In a related embodiment, detection of genes corresponding to the polynucleotides of the present invention can be conducted by primer extension reactions such as the polymerase chain reaction (PCR). To that end, PCR primers are utilized in pairs, as is well known, based on the nucleotide sequence of the gene to be detected. Preferably the nucleotide sequence is a portion of the nucleotide sequence of a polynucleotide of the present invention. Particularly preferred PCR primers can be derived from any portion of a DNA sequence encoding a polypeptide of the present invention, but are preferentially from regions which are not conserved in other cellular proteins.

Preferred PCR primer pairs useful for detecting the genes corresponding to the polynucleotides of the present invention and expression of these genes are described in the Examples, including the corresponding Tables. Nucleotide primers from the corresponding region of the polypeptides of the present invention described herein are readily prepared and used as PCR primers for detection of the presence or expression of the corresponding gene in any of a variety of tissues.

The diagnostic system includes, in an amount sufficient to perform at least one assay, a subject polypeptide of the present invention, a subject antibody or monoclonal antibody, and/or a subject nucleic acid molecule probe of the present invention, as a separately packaged reagent.

In another embodiment, a diagnostic system, preferably in kit form, is contemplated for assaying for the presence of the polypeptide of the present invention or an antibody

immunoreactive with the polypeptide of the present invention in a body fluid sample such as for monitoring the fate of therapeutically administered the polypeptide of the present invention or an antibody immunoreactive with the polypeptide of the present invention. The system includes, in an amount sufficient for at least one assay, a polypeptide of the present invention and/or a subject antibody as a separately packaged immunochemical reagent.

Instructions for use of the packaged reagent(s) are also typically included.

As used herein, the term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene or polycarbonate), paper, foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or antibody or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide or antibody have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody or antigen, respectively.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/ sample admixtures, temperature, buffer conditions and the like.

A diagnostic system of the present invention preferably also includes a label or indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

The word "complex" as used herein refers to the product of a specific binding reaction such as an antibody-antigen or receptor-ligand reaction. Exemplary complexes are immunoreaction products.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the

production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyante (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference. Other suitable labeling agents are known to those skilled in the art.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-amino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I. Another group of useful labeling means are those elements such as ^{11}C, ^{18}F, ^{15}O

and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such ^{111} indium or ^{3}H.

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species of the present invention.or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention. Exemplary specific binding agents are second antibody molecules, complement proteins or fragments thereof, S. *aureus* protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of the polypeptide of the present invention in a sample. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical

Publications of Los Altos. CA in 1982 and in U.S. Patents No. 3,654,090: No. 3,850,752; and No. 4,016,043. which are all incorporated herein by reference.

Thus, in some embodiments, an polypeptide of the present invention, an antibody or a monoclonal antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides can be used that are well known to those skilled in the art. Exemplary adsorption methods are described herein.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron (μm) to about 5 millimeters (mm) in diameter available from several suppliers, e.g., Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

Methods

While the various microglial subtypes may arise from the differentiation of cells from a common precursor pool that is possibly indistinguishable from that giving rise to the macrophage/dendritic cells, the roles played by differentiated microglia in normal neural physiology and neuropathology are determined in part by the ensembles of proteins that are expressed after differentiation. The studies were designed to identify and determine the microglial and macrophage transcripts that are regulated by an inflammatory response. The TOGA (Total Gene Analysis) method was used to identify digital sequence tags (DSTs) corresponding to mRNAs which concentrations differ between macrophage and microglia or that are induced in microglia by lipopolysaccharide (LPS) and gamma-interferon (IFNγ), two substances that initiate inflammatory responses when introduced into the CNS, and which elicit the induction of markers of inflammation when applied to microglial cells in culture.

Microglia were isolated from mixed glial cultures prepared from the brains of neonatal C57Bl/6J mice according to the method described in Raible et al., J.Neurosci. Res., 27:43-46 (1990). Briefly, CNS from newborn mice (postnatal day 1 to postnatal day 3) were stripped of meninges, mechanically dissociated, seeded into T-75 flasks and maintained in OM5 media supplemented with 10% FBS. After two to four weeks, cultures were trypsinized, resuspended in RPMI media supplemented with 10% FBS, but lacking phenol red and incubated in suspension for 60 minutes at 37°C to allow for the reexpression of trypsinized surface markers. Microglia were then purified by flow cytometry using PE-conjugated antibodies against FcR/CD16/CD32 (Pharmingen, San Diego, CA) as described in Carson et al., Glia, 22:72-85 (1998).

It is hypothesized that microglial responses may be different from those of peripheral macrophages, due at least in part to their interactions with other CNS cell types. Therefore, to study microglial activation, these cells were stimulated in the presence of astrocytes, oligodendrocytes and the other CNS cell types present in the mixed glial cultures for 22 hours with 50-100 ng/ml LPS and 10-100 U/ml IFNγ (Genzyme). Only after stimulation were microglia isolated by flow cytometry. LPS and IFNγ were chosen as global stimulators of microglial and macrophage function. LPS is a potent stimulator of several early events in

macrophage activation, including the production and secretion of TNFα, IL-1 and IL-6, and mimics bacterial sepsis such as that occurring in bacterial meningitis. When coupled with IFNγ, which is produced by activated TH1 T cells, microglia and macrophages express MHC class II, produce nitrogen and oxygen intermediates, and become fully tumoricidal.

In order to isolate a population of macrophages that had been stimulated to leave the bloodstream or other organs and infiltrate into a site of perceived pathology, peritoneal macrophages were prepared by standard methodologies. Briefly, macrophages were induced to migrate into the peritoneal cavity of C57BL/6 mice by the injection of Brewer's thioglycolate solution into the peritoneal cavity. Peritoneal macrophages were harvested at 3-5 days post-injection, by flushing the peritoneal cavities of halothane-euthanized mice with PBS. Macrophage cells were separated from contaminating nonadherent cells by their adherence to tissue culture plastic. Cells were either allowed to rest for 22 hours in culture or were stimulated with 50-100 ng/ml LPS and 10-100 U/ml IFNγ for 1 hour or 22 hours.

Cytoplasmic, polyA enriched RNA from each of four cell samples was prepared: unstimulated microglia, stimulated microglia, unstimulated peritoneal macrophage, stimulated macrophage. Standard methods of RNA isolation and polyA selection were used, according to the method described in Schiber et al., J. Mol. Biol., 142:93-116 (1980).

Isolated RNA was analyzed using a method of simultaneous sequence-specific identification of mRNAs known as TOGA (TOtal Gene expression Analysis) described in U.S. Patent No. 5,459,037 and U.S. Patent No. 5,807,680, hereby incorporated herein by reference. Preferably, prior to the application of the TOGA technique, the isolated RNA was enriched to form a starting polyA-containing mRNA population by methods known in the art. In a preferred embodiment, the TOGA method further comprised an additional PCR step performed using four 5' PCR primers in four separate reactions and cDNA templates prepared from a population of antisense cRNAs. A final PCR step that used 256 5' PCR primers in separate reactions produced PCR products that were cDNA fragments that corresponded to the 3'-region of the starting mRNA population. The produced PCR products were then identified by a) the initial 5' sequence comprising the sequence of the remainder of the recognition site of the restriction endonuclease used to cut and isolate the 3' region plus the sequence of the preferably four

parsing bases immediately 3' to the remainder of the recognition site, preferably the sequence of the entire fragment, and b) the length of the fragment. These two parameters, sequence and fragment length, were used to compare the obtained PCR products to a database of known polynucleotide sequences.

The method yields Digital Sequence Tags (DSTs), that is polynucleotides that are expressed sequence tags of the 3' end of mRNAs. DSTs that showed differential representation were selected for further study as candidates of activation-induced or microglial specific mRNAs. The intensities of the laser-induced fluorescence of the labeled PCR products were compared across sample isolated from treated and untreated microglia and macrophages.

Having generally described the invention. the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Example 1

Identification and Characterization of Activation-induced or Microglial-specific Polynucleotides

In general, double stranded cDNA is generated from polyA enriched cytoplasmic RNA extracted using an equimolar mixture of all 48 of a set of 5'-biotinylated anchor primers to initiate reverse transcription. One such suitable set is G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N (SEQ ID NO: 26), where V is A, C or G and N is A, C, G or T. One member of this mixture of 48 anchor primers initiates synthesis at a fixed position at the 3' end of all copies of each mRNA species in the sample, thereby defining a 3' endpoint for each species, resulting in biotinylated double stranded cDNA.

Each biotinylated double stranded cDNA sample was cleaved with the restriction endonuclease MspI, which recognizes the sequence CCGG. The resulting fragments of cDNA corresponding to the 3' region of the starting mRNA were then isolated by capture of the biotinylated cDNA fragments on a streptavidin-coated substrate. Suitable streptavidin-coated substrates include microtitre plates, PCR tubes, polystyrene beads, paramagnetic polymer beads

and paramagnetic porous glass particles. A preferred streptavidin-coated substrate is a suspension of paramagnetic polymer beads (Dynal, Inc., Lake Success, NY).

After washing the streptavidin-coated substrate and captured biotinylated cDNA fragments, the cDNA fragment product was released by digestion with NotI, which cleaves at an 8-nucleotide sequence within the anchor primers but rarely within the mRNA-derived portion of the cDNAs. The MspI-NotI fragments of cDNA corresponding to the 3' region of the starting mRNA, which are of uniform length for each mRNA species, were directionally ligated into ClaI-, NotI-cleaved plasmid pBC SK$^+$ (Stratagene, La Jolla, CA) in an antisense orientation with respect to the vector's T3 promoter, and the product used to transform *Escherichia coli* SURE cells (Stratagene). The ligation regenerates the NotI site, but not the MspI site, leaving CGG as the first 3 bases of the 5' end of all PCR products obtained. Each library contained in excess of 5 x 10^5 recombinants to ensure a high likelihood that the 3' ends of all mRNAs with concentrations of 0.001% or greater were multiply represented. Plasmid preps (Qiagen) were made from the cDNA library of each sample under study.

An aliquot of each library was digested with MspI, which effects linearization by cleavage at several sites within the parent vector while leaving the 3' cDNA inserts and their flanking sequences, including the T3 promoter, intact. The product was incubated with T3 RNA polymerase (MEGAscript kit, Ambion) to generate antisense cRNA transcripts of the cloned inserts containing known vector sequences abutting the MspI and NotI sites from the original cDNAs.

At this stage, each of the cRNA preparations was processed in a three-step fashion. In step one, 250ng of cRNA was converted to first-strand cDNA using the 5' RT primer (A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G, (SEQ ID NO: 27). In step two, 400 pg of cDNA product was used as PCR template in four separate reactions with each of the four 5' PCR primers of the form G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N (SEQ ID NO:28), each paired with an "universal" 3' PCR primer G-A-G-C-T-C-C-A-C-C-G-C-G-G-T (SEQ ID NO: 29).

In step three, the product of each subpool was further divided into 64 subsubpools (2ng in 20μl) for the second PCR reaction, with 100 ng each of the fluoresceinated "universal" 3' PCR

primer, the oligonucleotide (SEQ ID NO: 29) conjugated to 6-FAM and the appropriate 5' PCR primer of the form C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N (SEQ ID NO:30), using a program that included an annealing step at a temperature X slightly above the T_m of each 5' PCR primer to minimize artifactual mispriming and promote high fidelity copying. Each polymerase chain reaction step was performed in the presence of TaqStart antibody (Clonetech).

The products from the final polymerase chain reaction step for each of the tissue samples were resolved on a series of denaturing DNA sequencing gels using the automated ABI Prizm 377 sequencer. Data were collected using the GeneScan software package (ABI) and normalized for amplitude and migration. Complete execution of this series of reactions generated 64 product subpools for each of the four pools established by the 5' PCR primers of the first PCR reaction, for a total of 256 product subpools for the entire 5' PCR primer set of the second PCR reaction.

The mRNA samples from microglia and macrophages treated as described above were analyzed. Table 1 is a summary of the expression levels of 509 mRNAs determined from cDNA. These cDNA molecules are identified by their digital address, that is, a partial 5' terminus nucleotide sequence comprising the remainder of the MspI site and the four parsing bases for the 5' PCR primer of each subsubpool coupled with the length of the molecule, as well as the relative amount of the molecule produced in untreated microglia, treated microglia, untreated macrophages and treated macrophages. The 5' terminus partial nucleotide sequence is determined by the recognition site for MspI and the nucleotide sequence of the parsing bases of the 5' PCR primer used in the final PCR step. The length of the fragment was determined by interpolation on a standard curve.

For example, the entry in Table 1 that describes a DNA molecule identified by the ditigal address MspI GTTC 426, is further characterized as having a 5' terminus partial nucleotide sequence of CGGGTTC and a digital address length of 426 b.p. The DNA molecule identified as MspI GTTC 426 is further described as being expressed in control (2590) and activated (1650) microglia, but not control (153) or activated (185) macrophage cells (Table 1, Figure 1). Additionally, the DNA molecule identified as MspI GTTC 426 (clone MM_27) is described by its nucleotide sequence which corresponds with SEQ ID NO: 15.

Similarly, the other DNA molecules identified in Table 1 by their MspI digital addresses are further characterized by: (1) the level of gene expression in untreated microglia; (2) the level of gene expression in treated microglia: (3) the level of gene expression in untreated macrophages; and (4) the level of gene expression in treated macrophages.

Additionally, several of the isolated clones are further characterized as shown in Tables 2 and 3, and their nucleotide sequences are provided as SEQ ID NOs: 1-25. Several of the isolated clones described in Table 2 were also characterized by the level of gene expression in various tissues including lung, heart, kidney, liver, lymph nodes, spleen, testes and several brain tissues (cortex, midbrain, brainstem, and cerebellum).

The sequences of SEQ ID NO: 1-23 have had the MspI site found in the native state of the corresponding mRNA indicated by the addition of a "C" to the 5' end of the sequence. As noted above, the ligation of the sequence into the vector does not regenerate the MspI site; the experimentally determined sequence of the PCR products reported herein has C-G-G as the first bases of the 5' end.

The data shown in Figure 1 were generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-G-T-T-C, SEQ ID NO: 31) paired with the "universal" 3' primer (SEQ ID NO: 29) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

The results of TOGA analysis using the above-described 5' PCR primer with parsing bases GTTC (SEQ ID NO: 31) are shown in Figure 1, which shows the PCR products produced from mRNA extracted from (A) untreated microglia, (B) treated microglia, (C) untreated macrophages and (D) treated macrophages in four panels. The vertical index line indicates a PCR product of about 426 b.p. that is present in microglia, but not macrophage cells.

Some products, which were also differentially represented, appeared to migrate in positions that suggests that the products were novel based on comparison to data extracted from

GenBank. In these cases, the PCR product was isolated, cloned into a TOPO vector (Invitrogen) and sequenced on both strands. In order to verify that the clones isolated are from the same peak, PCR primers were designed based on the determined sequence and PCR was performed using the cDNA produced in the first PCR reaction as substrate. Oligonucleotides were synthesized corresponding to the 5' PCR primer in the second PCR step for each candidate extended at the 3' end with an additional 12-15 nucleotides from the sequences adjacent to the terminal MspI sites in the GenBank sequences. For example, for the 426 b.p. product disclosed above, the 5' PCR primer was G-A-T-C-G-A-A-T-C-C-G-G-G-T-T-C-A-A-C-C-G-C-G-T-G-A-A-G-G-T (SEQ ID NO: 55). This 5' PCR primer was paired with the fluorescent labeled 3' PCR primer (SEQ ID NO: 29) in PCRs using the cDNA produced in the first PCR reaction as substrate. The procedure was used to verify each candidate match to database entries. The results are shown in Table 3, below.

The products were separated by electrophoresis and the length of the clone was compared to the length of the original PCR product as shown in Figure 2. The upper panel (A) shows the PCR products produced using the original PCR primers, SEQ ID NO: 31 and SEQ ID NO: 29 (compare to the top panel in Figure 1A). In Figure 2B, the middle panel shows the length (as peak position) of the PCR product derived from the isolated clone as described above using the PCR primers, SEQ ID NO: 55 and SEQ ID NO: 29. In the bottom panel, Figure 2C, the traces from the top and middle panels are overlaid, demonstrating that the PCR product of the isolated and sequenced novel clone is the same length as the original PCR product.

As shown in Tables 2 and 3, MM_27 corresponds to a gene that encodes the G protein gamma-5 subunit. Interestingly, MM_27 is associated with control and activated microglia cells, but not macrophage cells, suggesting that its expression is specific to microglia. Thus, MM_27 from these results has the expected characteristics of a cell-specific marker useful to distinguish microglia from macrophage cells in CNS tissue.

Example 2

Identification and Characterization of Activation-induced or Microglial-specific Polynucleotides

Another example is shown in Figures 3 and 4. In Figure 3, a peak at about 244 is indicated, identified by digital address MspI GTTG 244 when a 5' PCR primer (SEQ ID NO: 32) was paired with SEQ ID NO: 29 to produce the panel of PCR products. The PCR product was cloned and sequenced as described in Example 1. To verify the identity of the isolated clone (SEQ ID NO: 14), oligonucleotides were synthesized corresponding to the 5' PCR primer in the second PCR step for each candidate extended at the 3' end with an additional 12-15 nucleotides from the sequences adjacent to the terminal MspI sites in the GenBank sequences. In this case the 5' PCR primer was G-A-T-C-G-A-A-T-C-C-G-G-G-T-T-G-C-A-C-C-T-A-T-T-G-C-A-T-G-T (SEQ ID NO: 54). This 5' PCR primer were paired with the fluorescently labeled 3' PCR primer (SEQ ID NO: 29) in PCRs using the cDNA produced in the first PCR reaction as substrate.

In Figure 4, the upper panel shows the PCR products produced using the original PCR primers, SEQ ID NO: 32 and SEQ ID NO: 29 (compare to Figure 3B). In Figure 4B, the middle panel shows the length (as peak position) of the PCR product derived from the isolated clone as described above. In the bottom panel Figure 4C, the traces from the top and middle panels are overlaid, demonstrating that the PCR product of the isolated and sequenced novel clone is the same length as the original PCR product.

As shown in Table 1, the DNA molecule identified by the digital address MspI GTTG 244 (clone MM_26), is further characterized as having a 5' terminus partial nucleotide sequence of CGGGTTG and a digital address length of 244 b.p. MM_26 is further described as being expressed at comparable levels in untreated macrophages (6242) and treated macrophages (6175). However, the treatment results in a marked regulation of the expression of MM_26 in microglia, producing a 26-fold increase between untreated microglia (45) and treated microglia (1180). MM_26 is further characterized by its nucleotide sequence which is presented in SEQ ID NO: 14.

The full-length gene comprising MM_26 is presently unidentified. Interestingly, MM_26 is associated with stimulated microglia cells and macrophage cells, but not with untreated microglia cells, suggesting its expression correlates with an activated phenotype.

Based on these results, MM_26 thus has the expected characteristics of a marker of an inflammatory response in the CNS.

EXAMPLE 3
Further Characterization of MM 3

The clone MM_3 (digital address AAGT 366) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-A-G-T; SEQ ID NO: 33) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The resultant 366 bp PCR product was isolated and characterized as described below. The sequence of the 317 bp insert (the balance being vector sequence) is given in SEQ ID NO: 18.

As shown in Table 1, the results of TOGA analysis indicate that MM_3 is present in microglia, but not macrophage cells. Additionally, MM_3 shows greater expression in microglia cells that have been stimulated with LPS/IFNγ than in unstimulated microglia cells. As shown in Table 2, the MM_3 clone corresponds with GenBank sequence U43086, which is identified as the mouse glucocorticoid-attenuated response gene 49 (GARG)/IRG2). In further characterization of DST 3, northern blot analyses were performed: 1) to determine the pattern of expression in various tissues and cells and 2) to determine differences in expression between unstimulated and stimulated microglia and macrophage cells.

Poly A enriched mRNA was prepared from whole brain isolated from either neonatal (post-natal day 1, P1) or adult mice, microglia isolated from mixed glial cultures, peritoneal macrophage cells, kidney fibroblasts, and bone marrow-derived dendritic cells. The glial and macrophage cells were prepared according to the previously described methods. Whole brain was prepared by rapidly sacrificing mice using halothane inhalation, immediately removing the brain from the skull, and homogenizing the whole brain in preparation for RNA extraction.

Kidney fibroblast cells were isolated from adult C57BL/6 mice (both wild-type and relB knock out mice) as described in Feng et al., Am. J. Phys., 266:F713-F722 (1994). Briefly, cell

suspensions were prepared from kidney immediately after removal from halothane euthanized mice. Adherent cells were cultured for 15 passages, at which time the cultures consisted of only fibroblast cells.

Dendritic cells were isolated from bone marrow according to the method described in Talmor et al., Eur. J. Imm., 28:811-817 (1998). Briefly, marrow from femurs was eluted in RPMI 1640 tissue culture media. Cells were recovered by centrifugation and plated at one mouse equivalent per 150mm tissue culture plate in RPMI 1640 supplemented with 10% fetal bovine serum, 25mM Hepes, 1mM glutamine, 50 μM 2-mercaptoethanol, 50 U/ml granulocyte/macrophage colony stimulating factor and 100 U/ml interleukin-4. After 2 days, non-adherent cells were transferred to a new 150mm tissue culture plate. Five days after initiation of bone marrow cultures, the cells were plated in AIM V media (Gibco/BRL) and 48 hours later , dendritic cells were isolated from the non-adherent population in both 150mm plates by flow cytometry. Dendritic cells were identified by size, side scatter and high B7.2 expression using fluorescein isothiocyanate –conjugated antibodies against B7.2.

The glial and macrophage cells were either unstimulated, stimulated for 1 hour with LPS/IFNγ (50ng/ml LPS; 10 U/ml IFNγ), or stimulated for 22 hours with LPS/IFNγ (50ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation. The kidney fibroblasts isolated from wild-type and rel B knock out (KO) mice were both treated with LPS (50 ng/ml) for 2 hours. Dendritic cells were not treated.

In addition, poly A enriched mRNA was prepared from various freshly-isolated murine tissues including lung, heart, kidney, liver, spleen, lymph node, testis, and several regions of the brain (cortex, midbrain, brainstem, and cerebellum). The various tissues were isolated by rapidly sacrificing mice using halothane inhalation, immediately removing the specified tissue and placing it in ice-cold phosphate buffered saline (PBS) prior to homogenation. The brain regions were isolated by rapidly removing the brain from the skull, separating the cortex, midbrain, brainstem, and cerebellum regions and placing them in separate tubes of ice cold PBS prior to homogenation. The cytoplasmic RNA and poly A enriched mRNA was prepared from each of these tissues using the method described in Schiber et al., J. Mol. Biol., 142:93-116 (1980).

Shown in Figure 5A-C, northern blot analyses were performed using 2 μg of poly A enriched mRNA extracted from the indicated tissues and cells, as described above. The mRNA transcripts were fractionated by electrophoresis on a 1.5% agarose gel containing formaldehyde, transferred to a biotrans membrane, and prehybridized for 30 minutes in Expresshyb (Clonetech). 25-100 ng of MM_3 insert DNA (prepared by Eco RI restriction digest of the vector) was labeled with [α-^{32}P]-dCTP by oligonucleotide labeling to specific activities of approximately 5 x 10^8 cpm/μg and added to the prehybridization solution and incubated for 1 hour. Filters were washed to high stringency (0.2X SSC) (1 X SSC: 0.015 M NaCl and 0.0015 M Na citrate) at 68°C and then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) for up to one week.

As can be seen in Figure 5A, the transcript detected using MM_3 is expressed at a significantly higher level in mixed glial cultures than in whole brain from either neonatal or adult mice. Likewise, the expression is significantly higher in mixed glial cultures than in macrophages and kidney fibroblasts. In response to LPS/IFNγ stimulation (50ng/ml LPS; 10 U/ml IFNγ), the expression continues to increase in mixed glial cultures exposed to LPS/IFNγ for 22 hours, while its expression in peritoneal macrophages has already begun to decline after 22 hours.

Also, the expression of the transcript detected by MM_3 in LPS treated fibroblasts was not affected by the absence of relB in knock out mice. RelB is a subunit of the NF-kappa B transcription factor. LPS-stimulated kidney fibroblasts express numerous chemokines and inflammatory molecules. Given that mice lacking the relB gene can not turn off the expression of these molecules, the use of such mice allows the detection of inflammatory molecules that are normally expressed at levels below the level of detection.

Figure 5B shows microglia, macrophage, and dendritic cells that were either untreated, treated with LPS/IFNγ (50ng/ml LPS; 10 U/ml IFNγ), or LPS alone (50ng/ml) for 22 hours prior to RNA isolation. As can be seen, a transcript detected using MM_3 is inducible in both microglia and macrophages in response to stimulation, although the expression is substantially higher in microglia in both control and LPS/IFNγ stimulated cells. The expression of this transcript was not detected in dendritic cells.

As shown in Figure 5C, the transcript detected using MM_3 is expressed in kidney, liver, lymph node, and various regions of the brain (midbrain, brainstem, and cerebellum), although expression in the brain and lymph node is minimal. MM_3 is not expressed in the lung, heart, spleen, testis, or cortex.

The northern blot analyses revealed an mRNA of about 2-3 kb (data not shown) which corresponds to the mouse glucocorticoid-attenuated response gene 49 (GARG)/IRG2. This gene has been described in murine Swiss 3T3 (Smith et al., Arch. Biochem. Biophys., 330:290-300 (1996)) and RAW 264.7 cell lines (Lee et al., J. Immunol., 152:5758-5767 (1994)) as a gene that is absent in nonstimulated macrophages, but expressed after LPS or IFNγ treatment. Further, the induction of gene expression is suppressed by treatment with glucocorticoid hormones, such as dexamethasone. Although the function of the protein is not known, it is believed that genes induced by LPS or IFNs have a role in the cellular responses to bacterial infections. For example, Lee et al. has hypothesized that the protein plays a role in substrate conversion or sequestration that might be significant in antimicrobial or antiviral activity (Lee et al. (1994)). Others have hypothesized that GARGs function in intercellular rather than intracellular processes (Smith et al., J. Biol. Chem., 270, 16756-16765 (1995)).

Smith et al. has described the GARG gene product, which is a 43 amino acid protein of about 47,200 D. The protein has multiple tetratricopeptide repeat (TPR) domains, which are loosely conserved 34 amino-acid residue repeat units involved in specific protein-protein interactions, including apoptosis-dependent ubiquitination of cyclin B, transcriptional repression, and protein import into peroxisomes and mitochondria (Goebel et al., Trends Biochem. Sci., 16:173-177 (1991)). Smith et al. suggest that the LPS and IFN-induced GARG/IRG2 protein is a regulatory factor that participates in the formation of multicomponent assemblies, whereby the individual TPR domains mediate the cellular responses to interferons and LPS by regulating the formation of the multicomponent assemblies.

While the expression of GARG/IRG2 has been described in other tissues, the present invention provides novel data regarding the expression of GARG/IRG2 in microglia. Specifically, the present results show that GARG/IRG2 gene expression is enriched in microglia

as compared to macrophages and is up-regulated by LPS/IFNγ to a much higher extent and with greatly sustained kinetics in microglia as compared to macrophages. Based on these results which indicate that the GARG/IRG2 gene is associated primarily with the activated state, MM_3 has the characteristics of a marker for activated microglial cells. For example, labeled MM_3 or fragments thereof can be used as probes for northern blots and *in situ* hybridization to indicate activated microglia. Also, translations of MM_3 ("MM_3 peptides") can be used to make antibodies that are useful for identifying corresponding polypeptides in techniques such as western blotting, immunocytochemistry ,and ELISA assays using standard techniques such as those described in U.S. Patent No. 4,900,811, incorporated by reference herein.

Additionally, MM_3 could be useful as a therapeutic agent, given its hypothesized role as an antibacterial or antiviral protein, or a mediator of the cellular response to LPS/IFNγ.

EXAMPLE 4
Further Characterization of MM_11

The clone MM_11 (digital address AGGT 315) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5' –PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-G-G-T; SEQ ID NO: 34) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The insert, when sequenced, had the sequence presented as SEQ ID NO: 1.

As shown in Table 1, the results of TOGA analysis indicate that MM_11 is differentially expressed in treated versus untreated microglia and macrophage cells. As shown in Table 2, the MM_11 clone corresponds with GenBank sequence AA543723, which is of unknown identity. Northern Blot analyses were performed to determine the pattern of expression in unstimulated and stimulated microglia, macrophage, and dendritic cells.

Total RNA was prepared from microglia isolated from mixed glial cultures, peritoneal macrophage cells, and bone marrow-derived dendritic cells, as described in Example 3. The microglia and macrophage cells were either unstimulated or stimulated for 22 hours with LPS/IFNγ (50ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation. The dendritic cells were not

stimulated prior to RNA isolation. The cytoplasmic RNA was isolated from the various tissues and cells using the method described in Example 3. Shown in Figure 6, northern blot analyses were performed as described in Example 3, except that 10 μg of total RNA was loaded into each lane and MM_11 insert DNA was radiolabeled and used as a probe.

Figure 6 shows that the transcript detected using MM_11 is enriched in microglia as compared to macrophage and dendritic cells. Further, MM_11 is dramatically reduced after either LPS stimulation or LPS/IFNγ stimulation. While low level of expression of MM_11 can be detected in macrophage and dendritic cells, the expression of MM_11 is more than 20-fold greater in microglial cells. The expression of MM_11 is repressed in both microglia and macrophages after 22 hour of LPS/IFNγ treatment.

At present, the full-length transcript detected using MM_11 is of unknown identity, but matches an EST in the GenBank database. Preliminary size analysis indicates that the transcript is approximately 1 kb in size (data not shown). The observation that MM_11 is abundantly expressed in unstimulated microglia, but not macrophage or dendritic cells suggests that the MM_11 gene product may be useful as a neural-specific marker by which to identify microglia. For example, labeled MM_11 or fragments thereof can be used as probes for northern blots and *in situ* hybridization to differentiate microglia from macrophage cells in the CNS. Translations of MM_11 ("MM_11 peptides") can be used to make antibodies that are useful for identifying corresponding polypeptides in techniques such as western blotting, immunocytochemistry ,and ELISA assays using standard techniques as described above.

Interestingly, MM_11 is down-regulated in LPS/IFNγ-stimulated microglia, suggesting that this molecule could be regulated by a cytokine or other agent involved in the inflammatory response.

EXAMPLE 5
Further Characterization of MM 12

The clone MM_12 (digital address ACAA 381) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-C-A-A; SEQ ID NO: 35) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The resultant 381bp PCR product was isolated and characterized as described below. The sequence of the insert is given by SEQ ID NO: 2.

As shown in Table 1, the results of TOGA analysis indicate that MM_12 is differentially expressed in microglia and macrophage cells. As shown in Table 2, the MM_12 clone corresponds with GenBank sequence U25096, which is identified as the mouse Kruppel-like factor (LKLF). Northern blot analyses were performed: 1) to determine the pattern of expression in various tissues and cells and 2) to determine differences in expression between unstimulated and stimulated microglia, macrophage, and dendritic cells.

Total RNA was prepared from the following murine cell cultures: microglia isolated from murine mixed glial cultures, peritoneal macrophage cells, and bone marrow-derived dendritic cells. The microglial, macrophage, and dendritic cells were prepared according to the previously described methods in Example 3. The glial and macrophage cells were either unstimulated or stimulated for 22 hours with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation. Dendritic cells were not treated.

In addition, poly A enriched mRNA was prepared from a variety of freshly-isolated murine tissues, including lung, heart, kidney, liver, spleen, lymph node, testis, and several regions of the brain (cortex, midbrain, brainstem, and cerebellum). The total RNA and poly A enriched mRNA was prepared as described in Example 3. Northern blot analyses were performed according to the method described in Example 3, using either 10μg of total RNA (Figure 7A) or 2μg of poly A enriched mRNA (Figure 7B). The MM_12 insert DNA was radiolabeled and used as the oligonucleotide probe.

Shown in Figure 7A, a transcript detected using MM_12 shows greater expression in microglia than in macrophages or dendritic cells. Furthermore, its expression in microglia is slightly reduced after 22 hour LPS/IFNγ treatment. In contrast, the expression of the transcript is increased LPS/IFNγ- stimulated macrophage cells compared with unstimulated cells. Longer exposure of the northern blot reveals very weak expression of the transcript in both the unstimulated macrophages and dendritic cells.

As shown in Figure 7B, the transcript detected using MM_12 is expressed in most adult mouse tissues. However its expression is most abundant in lymph node, lung and heart.

The transcript detected by MM_12 is approximately 1.5-3 kb as determined by preliminary size analysis and corresponds with an identified Kruppel-like factor (LKLF) gene. The LKLF gene is a zinc-finger transcription factor gene (Anderson et al., Mol. Cell Biol., 15:5957-5965 (1995)). Such factors bind to regulatory regions of the DNA, influencing the transcriptional activity of the gene. The LKLF gene has been shown to be developmentally regulated with discrete patterns of expression in different tissues. Anderson et al. reports that the highest level of LKLF expression is in lung tissue, with reduced levels found in spleen, skeletal muscle, testes, heart and uterus.

Although LKLF expression has been characterized in various mouse and rat tissues, its expression in lymph node. microglia, macrophages or dendritic cells has not been previously examined. Interestingly, the results of the present study show that LKLF is highly expressed in both microglia and lymph nodes. Based on these results which indicate that the level of LKLF expression is significantly higher in unstimulated microglia as compared to unstimulated macrophage, MM_12 has the characteristics of a marker for microglial cells in normal or unactivated CNS. As discussed in the above examples, labeled MM_12 or fragments thereof can be used as probes for northern blots and *in situ* hybridization to differentiate microglia from macrophage cells in the CNS. Translations of MM_12 ("MM_12 peptides") can be used to make antibodies that are useful for identifying corresponding polypeptides.

EXAMPLE 6
Further Characterization of MM_14

The clone MM_14 (digital address TATA 249) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-A-T-A; SEQ ID NO: 36) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The sequence determined for the insert is given in SEQ ID NO: 4.

As shown in Table 1, MM_14 is expressed in control (1753) and stimulated (1610) macrophages, but not in control (32) or stimulated (113) microglial cells. MM_14 corresponds with GenBAnk sequence X80937, which is identified as a sequence found in the mouse Ral interacting protein (RIP1). RIP1 is believed to be involved in intracellular signaling along G-mediated pathways, based on data which shows that RIP1 binds to Ral in a GTP-dependent manner. The Ral protein is one of a large family of low molecular weight GTPases, the most well-known of which is Ras. Ral is a 206 amino acid protein which shares greater than 50% homology with Ras. The Ral proteins are the major GTP binding protein in human platelets and are also abundant in the supernatant fraction of rabbit and bovine brains. Park et al. reports that RIP1 is expressed in a wide variety of tissues, including ovaries, skeletal muscle, heart, brain, lung, kidney, liver and spleen (Park et al., Oncogene, 11:2349-2355 (1995)).

Interestingly, the data presented in Table 1 indicates that RIP1 is not expressed in microglial cells. The differential expression between microglia and macrophages illustrates that microglial and macrophages are physiologically distinct. Further, given the differential expression, MM_14 may be useful as a marker to differentiate between microglia and macrophage cells in CNS tissue.

EXAMPLE 7

Further Characterization of MM 18

The clone MM_18 (TTGG 262) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-T-G-G; SEQ ID NO: 37) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus.

PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The resultant 262 bp PCR product was isolated and characterized as described below. The sequence of the insert is given in SEQ ID NO: 8.

As shown in Table 1, the results of TOGA analysis indicate that MM_18 is differentially expressed in microglia and macrophage cells. As shown in Table 2, the MM_18 clone corresponds with GenBank sequence X67319, which is identified as the GOLLI-MBP/transcript overlapping myelin basic protein. Northern blot analyses were performed to determine the pattern of expression in various tissues and cells and to determine differences in expression between unstimulated and stimulated microglia and macrophage cells.

Poly A enriched mRNA was prepared from microglia isolated from murine mixed glial cultures and peritoneal macrophage cells, as previously described. The microglial and macrophage cells were isolated according to the previously described methods in Example 3. The microglia and macrophage cells were either unstimulated or stimulated for 1 hour or 22 hours with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation.

In addition, poly A enriched mRNA was prepared from a variety of freshly-isolated murine tissues, including lung, heart, kidney, liver, spleen, lymph node, testis and tissues isolated from several regions of the brain (cortex, midbrain, brainstem, and cerebellum). The poly A enriched mRNA was prepared as described in Example 3. Northern blot analyses were performed according to the method described in Example 3, using 2μg of poly A enriched mRNA. The MM_18 insert DNA was radiolabeled and used as the oligonucleotide probe.

The MM_18 probe detected four distinct mRNA transcripts ranging in size from about 1-2 kb to about 6 kb which can be seen in both Figures 8A and 8B. Shown in Figure 8A, transcripts 2 and 3 are present in both microglia and macrophage cells. Interestingly, transcript 3 is strongly up-regulated following LPS/IFNγ treatment. In macrophage cells, the up-regulation is strongest after 1 hour and decreases after 22 hours. Also, transcript 2 is present only in stimulated microglia and macrophage cultures, suggesting that it is induced by LPS/IFNγ

treatment. Transcripts 1 and 4 are present in both microglia and macrophage cells, but show greater level of expression in macrophages.

As shown in Figure 8B, transcripts 2 and 3 are present in all tissues examined (transcripts 2 and 3 are visible in lung and spleen samples upon long exposure). In brain sections, transcript 1 is also easily detected. Transcript 4 is present in highest abundance in lymph node and testis, exhibiting a lower level of expression in other tissues. A longer exposure of the northern blot revealed that transcript 4 is expressed at the same levels as transcripts 2 and 3 in the spleen. Transcript 3 is the major transcript detected in testes. Additional northern blots not shown reveal that the expression of transcripts 2, 3, and 4 in the spleen was the same in neonatal (1 day post-natal) and adult tissue. Similarly, the expression of transcripts 2 and 3 in brain was the same in neonatal (1 day post-natal) and adult tissue.

MM_18 corresponds to the 3' end of exon 5c of the GOLLI-myelin basic protein (GOLLI-MBP) gene. The MBP gene was shown to be composed of overlapping genes and subsequently termed the GOLLI-MBP gene. The GOLLI-MBP gene encodes for two families of proteins which include the classic MBPs (consisting of six isoforms), and the GOLLI-MBPs, (consisting of three isoforms). Two of the GOLLI-MBP isoforms (J37 and BG21) contain sequences which are in frame with and thus share sequences in common with the classic MBPs. Thus, in J37 and BG21, GOLLI-MBP exons 5a and 5b correspond to exons 1a and 1b of the classic MBP (there is no exon 1c which corresponds to exon 5c in the classic MBP). A description of the various mRNAs and principal protein products of the GOLLI-MBP gene is found in Voskuhl, R., Imm. Rev., 164: 81-92 (1998), which is incorporated in its entirety by reference herein (see also, Grima et al., J. Neurochem., 59:2318-2323 (1992)). Figure 9 provides a schematic diagram of MM_18 in relation to the GOLLI-MBP gene transcripts.

MBP is one of the major structural proteins of CNS myelin. Autoimmune attacks directed against MBP induce multiple sclerosis-like symptoms in animal models. The discovery that GOLLI-MBP is expressed by cells of the immune system suggests that the expression of this molecule may play a role in either preventing autoimmune attacks against myelin under normal, healthy conditions or inducing or aggravating autoimmune attacks against CNS myelin under neurodegenerative conditions.

Previous studies of GOLLI-MBP expression detected only a single 5.1 kb transcript containing exon 5c. However, using the present inventive MM_18 sequence as a probe allowed the detection and identification of three previously unreported transcripts of GOLLI-MBP containing exon 5c, ranging in size from about 1.5 kb to about 6 kb. Transcripts 2 and 3, one of which is novel, were present in all tissues screened. Novel transcript 4 was present in the lymph nodes, spleen and testes. Novel transcript 1 was specific to CNS tissue. The present data also show that the novel transcripts are transiently up-regulated by LPS/IFNγ in macrophage cells. These data suggest that GOLLI-MBP could play a significant role in CNS autoimmune disease.

EXAMPLE 8

Further Characterization of MM 20

The clone MM_20 (digital address TGTG 411) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-G-T-G; SEQ ID NO: 38) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The resultant 411 bp PCR product was isolated and characterized as described below. The sequence of the insert is given in SEQ ID NO: 10.

As shown in Table 1, the results of TOGA analysis indicate that MM_20 is differentially expressed in microglia and macrophage cells. As shown in Table 2, the MM_20 clone corresponds with GenBank sequence MMGSHPX, which is identified as glutathione peroxidase. Northern Blot analyses were performed to determine the pattern of expression in various tissues and cells and to determine differences in expression between unstimulated and stimulated microglia and macrophage cells.

Poly A enriched mRNA was prepared from microglia isolated from murine mixed glial cultures and peritoneal macrophage cells, as previously described. The microglial and macrophage cells were isolated according to the previously described methods in Example 3.

The microglia and macrophage cells were either unstimulated or stimulated for 1 hour or 22 hours with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation.

In addition, poly A enriched mRNA was prepared from freshly-isolated murine spleen and brain tissues of neonatal (postnatal day 1, P1) and adult mice. The poly A enriched mRNA was prepared as described in Example 3. Northern blot analyses were performed according to the method described in Example 3, using 2μg of poly A enriched mRNA. The MM_20 insert was radiolabeled and used as the oligonucleotide probe.

Figure 10 shows that a transcript detected using MM_20 is present in spleen, brain, microglial cells and macrophages. The level of expression is higher in both neonatal and adult spleen tissue than in the corresponding brain tissues. Interestingly, the expression of the transcript in both microglia cells and macrophage cells is down-regulated following 22 hour treatment with LPS/IFNγ.

The transcript detected using MM_20 is approximately 2-3 kb in size as determined by preliminary size analysis and corresponds with the enzyme glutathione peroxidase. Glutathione peroxidase is believed to play an important protective role under conditions of oxidative stress. Excitotoxic processes in the brain which occur under conditions of stroke and primary neurodegenerative diseases are accompanied by an excessive formation of reactive oxygen intermediates, such as superoxide and other oxygen free radicals. Superoxide dismutase catalyzes the removal of oxygen from superoxide, resulting in the generation of peroxides which are then removed enzymatically by catalase and glutathione peroxidase.

Studies comparing the distribution pattern of cellular glutathione peroxidase in normal CNS tissue versus excitotoxically induced CNS tissue, reveal differences in the distribution pattern of glutathione peroxidase depending on the activation state (Lindenau et al., Glia, 24:252-256 (1996)). In normal CNS, glutathione peroxidase is expressed primarily in the microglia located in all CNS regions. However, in activated CNS, glutathione peroxidase is expressed primarily in the microglia at the site of the degenerative lesion and also expressed in astrocytes located in tissue surrounding the lesion core. Other researchers have found increased

glutathione expression in activated astroglia and macrophages found in infarcted areas of the human brain (Takizawa et al., J. Neurol. Sci., 122:66-73 (1994)).

Among other things, these results indicate that MM_20 can be useful as a marker of glutathione peroxidase expression in these and other instances.

EXAMPLE 9

Further Characterization of MM 21

The clone MM_21 (digital address TCAT 410) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5' –PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-C-A-T; SEQ ID NO: 39) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software. The resultant 410 bp PCR product was isolated and characterized as described below. The sequence of the insert is given in SEQ ID NO: 11.

As shown in Table 1, the results of TOGA analysis indicate that MM_21 is differentially expressed in microglia and macrophage cells. As shown in Table 2, the MM_21 clone corresponds with GenBank sequence AA183527 which is presently unidentified. Northern blot analyses were performed to determine the pattern of expression in various tissues and cells and to determine differences in expression between unstimulated and stimulated microglia and macrophage cells.

Poly A enriched mRNA was prepared from microglia isolated from murine mixed glial cultures and peritoneal macrophage cells, as previously described. The microglial and macrophage cells were isolated according to the previously described methods in Example 3. The microglia and macrophage cells were either unstimulated or stimulated for 1 hour or 22 hours with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation.

In addition, poly A enriched mRNA was prepared from freshly-isolated murine spleen and brain tissues of postnatal day 1 (P1) and adult mice, as well as from murine lung, heart, kidney, liver, lymph nodes, testes and brain tissue (cortex, midbrain, brainstem, cerebellum). The poly A enriched mRNA was prepared as described in Example 3. Northern blot analyses were performed according to the method described in Example 3, using 2μg of poly A enriched mRNA. The MM_21 insert was radiolabeled and used as the oligonucleotide probe.

Figure 11A shows that the expression of the transcript detected by MM_21 is very restricted. The transcript is present in 1 hour stimulated macrophages, but not in microglia cells. Furthermore, the transcript is not expressed in spleen or brain tissue. Interestingly, in macrophage cells, expression of the detected transcript is strongly up-regulated within 1 hour of LPS/IFNγ exposure. However, this up-regulation is transitory, as the expression is negligible after 22 hour exposure to LPS/IFNγ. Notably, several transcripts are detected in the 1 hour treated macrophages, corresponding to sizes greater than 7 kb. The data shown in Figure 11B further indicate that the expression is tissue-specific. MM_21 is not expressed in any of the tissues tested except lymph node, where it shows strong expression.

The results shown in Figures 11A and 11B indicate that the expression of the transcript detected by MM_21 is tissue-specific and highly regulated. The observation that the expression of this transcript is limited to the lymph nodes is interesting. During inflammatory responses, T- and B-lymphocytes, as well as macrophages and dendritic cells, infiltrate a tissue site and organize into structures that resemble lymph nodes. The formation of neo-lymph nodes at the site of inflammation is a feature not only of many CNS diseases, such as multiple sclerosis, but also of peripheral inflammatory diseases, such as type I juvenile autoimmune diabetes (Lo, et al., Immunol. Rev. 169:225-239 (1999)). Activation of stromal tissue and/or infiltrating macrophages has been speculated to induce neo-lymph node formation at the site of inflammation.

The transcript detected by MM_21 is one of the first examples of a molecule with such restricted tissue expression. Thus MM_21 can be a diagnostic indicator of early autoimmune or of early inflammatory disease and lymph node formation. For example, labeled MM_21 or fragments thereof can be used as probes for northern blots and *in situ* hybridization to detect an

inflammatory or autoimmune response. Translations of MM_21 ("MM_21 peptides") can be used to make antibodies that are useful for identifying corresponding polypeptides.

EXAMPLE 10

Further Characterization of MM_23

The clone MM_23 (digital address TCGG 314) was obtained using the above-described TOGA analysis methods. The TOGA data was generated with a 5'–PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-C-G-G; SEQ ID NO: 40) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software The resultant 314 bp PCR product was isolated and characterized as described below. The sequence of the insert is given in SEQ ID NO: 13.

As shown in Table 1, the results of TOGA analysis indicate that MM_23 is differentially expressed in microglia and macrophage cells. As shown in Table 2, the MM_23 clone corresponds with GenBank sequences C80966, AF150087, and AF165967, one of which is a DDP-like molecule that is the homologue to yeast protein TIM10 (AF165967). Northern Blot analyses were performed to determine the pattern of expression in various tissues and cells and to determine differences in expression between unstimulated and stimulated microglia and macrophage cells.

Poly A enriched mRNA was prepared from microglia isolated from murine mixed glial cultures and peritoneal macrophage cells, as previously described. The microglial and macrophage cells were isolated according to the previously described methods in Example 3. The microglia and macrophage cells were either unstimulated or stimulated for 1 hour or 22 hours with LPS/IFNγ (50 ng/ml LPS; 10 U/ml IFNγ) prior to RNA isolation. In addition, poly A enriched mRNA was prepared from freshly-isolated murine spleen and brain tissues of neonatal (postnatal day 1, P1) and adult mice, as well as from murine lung, heart, kidney, liver, lymph nodes, testes and brain tissues (cortex, midbrain, brainstem, cerebellum). The poly A enriched mRNA was prepared as described in Example 3. Northern blot analyses were performed according to the method described in Example 3, using 2μg of poly A enriched mRNA. The MM_23insert was radiolabeled and used as the oligonucleotide probe.

Figures 12A shows that a transcript detected by MM_23 is expressed in microglial cells and is up-regulated by 22 hour exposure to LPS/IFNγ. Longer exposure northern blots show that the transcript is expressed in macrophage cells that have been exposed to LPS/IFNγ for 1 hour or 22 hours (Figure 12C). Additionally, the transcript is present in both neonatal and adult brain tissue, exhibiting slightly higher expression in adult tissue. The detected transcript is also expressed at very low levels in spleen. Figure 12B shows that the transcript is widely expressed in a variety of tissues, including the cortex, midbrain, brainstem, cerebellum, heart, kidney, liver, and testis. It is also expressed at lower levels in the lung and lymph nodes.

The human homologue of the molecule detected with MM_23 has been cloned and entered directly into GenBank as a DDP-like molecule that is the homologue to the yeast protein TIM10. To date, the cloning and characterization of this human molecule has not been published in a journal article. In yeast, TIM10 has been identified as a mitochondrial protein encoded by the cellular (but not the mitochondrial) genome. The expression of this molecule has not been well-characterized outside of the yeast system.

The transcript detected with MM_23 is about 0.7 kb in size and shares significant homology at the amino acid level to the deafness/dystonia peptide (DDP) gene. The DDP gene is believed to encode an evolutionarily conserved novel polypeptide necessary for normal human neurological development. The DDP gene was originally identified through positional and deletion studies where the absence of the DDP sequence was associated with deafness, dystonia, and mental deficiency (Jin et al., Nature Genetics, 14:177-180 (1996)). The clinical findings in DDP and related disorders suggest a progressive neurodegenerative disorder affecting the central nervous system, basal ganglia, corticospinal tract and possibly the brain stem. DDP contains two exons and a single intron of approximately 2 kb. Interestingly, a 1.2 kb DDP transcript has been detected in a range of adult and fetal tissues, including skeletal muscle, heart and brain, showing highest levels of expression in fetal and adult brain.

The predicted 97 amino acid DDP protein has a molecular weight of 11 kD. As shown in Figure 13, DDP exhibits high similarity with a predicted 11.4 kD protein from the fission yeast *S. pombe* based on exon predictions from genomic sequence (GenBank Z54308, gene

SPAC13G6.04). The predicted yeast protein has 98 amino acids with high similarity to DDP over 63 amino acids (40% identity; 60% similarity). A second predicted polypeptide translated from the EST yv59a08.s1 (GenBank N57799) also has similarity to DDP over 64 amino acids (42% identity; 62% similarity). Likewise, MM_23 shares significant similarity with the DDP protein (41% identity).

Similar to the results obtained by Jin et al., the present analyses reveal that the transcript detected by MM_23 is highly expressed in fetal brain and continues to be highly expressed in adult brain, suggesting that it may be involved in neurological development. While the present data indicate that this molecule is expressed at a low level in a variety of tissues, it is enriched in microglia as compared to macrophage cells. The differential expression of this molecule again reveals sustained physiological differences between microglia and macrophages. Further, given the differential expression it is possible that MM_23 could be useful as a marker to differentiate between microglia and macrophages in normal CNS.

The MM_23 sequence shown in Figure 14 (SEQ ID NO: 67) has been cloned into an expression vector. The open reading frame of MM_23 was amplified under high fidelity PCR conditions, using a pfu:Taq polymerase unit ratio of 2:1 and primers ATGGCCGAGCTTGGTGAAGCGGAC (SEQ ID NO: 68) and CTGCCCTCCTTTCTGTACGATCTG (SEQ ID NO: 69), in which the former contains the MM_23 initiator methionine triplet. The PCR product was isolated from a preparative gel, TA cloned into pBAD-TOPO (Invitrogen, Carlsbad, CA), and used to transform TOP10 E. *coli* according to the method described in U.S. Patents 5,487,993 and 5,766,891 and Shuman, S., J. Biol. Chem. 269: 32678-32684 (1994). Plasmid was isolated from a single transformant and the sequence of its insert was determined and found to be identical to the MM_23 open reading frame. A map of the pBAD-TOPO expression vector is shown in Figure 15.

The described MM_23 expression vector can be used to generate protein for functional studies and for the production of MM_23-specific antibodies. MM_23 can be used in research and diagnostic testing to monitor the presence of DDP and related gene products. For example, labeled MM_23 or fragments thereof can be used as probes for northern blots and *in situ* hybridization. Translations of MM_23 ("MM_23 peptides") can be used to make antibodies that

are useful for identifying corresponding polypeptides in techniques such as western blotting, immunocytochemistry ,and ELISA assays using standard techniques such as those described in U.S. Patent No. 4,900,811.

The foregoing is intended to be illustrative of the present invention, but not limiting. Numerous variations and modifications of the present invention may be effected without departing from the true spirit and scope of the invention.

Table 1					
Digital Address (Msp1)		Microglia Untreated	Microglia Treated	Macrophage Untreated	Macrophage Treated
AAAG	449	1533	1517	586	352
AAAT	113	728	197	61	48
AACA	126	1806	1379	166	311
AACA	131	1019	613	99	96
AACA	395	1231	579	46	94
AACC	275	379	290	60	39
AACC	408	218	179	23	38
AACT	364	1389	3820	80	202
AACT	392	914	388	93	83
AAGA	228	181	241	35	39
AAGC	498	4346	4076	315	228
AAGG	394	1317	863	197	255
AAGG	409	474	442	34	64
AAGT	196	2260	598	1710	595
AAGT	366	502	1637	58	128
AATA	97	1198	850	119	62
AATT	107	1920	1144	281	244
ACAC	342	181	856	36	25
ACAC	485	209	271	38	42
ACAG	470	695	361	18	85
ACAA	381	1135	400	2420	184
ACCA	496	524	307	50	28
ACCC	112	2347	2543	139	94
ACCC	457	215	208	37	40
ACCG	463	216	269	57	83
ACGA	76	766	905	132	151
ACGC	401	203	163	26	25
ACGG	408	2709	789	60	74
ACGT	347	96	2377	1462	3340
ACGT	366	296	712	48	59
ACGT	392	658	253	67	66
ACTT	293	3251	3422	113	176
AGCA	90	1141	765	134	82
AGCA	220	4583	4033	747	1003
AGCG	400	2218	2511	229	70
AGGC	300	262	305	71	82
AGTC	109	499	621	87	59
AGTC	323	1610	113	318	70
AGTT	435	162	279	48	69
AGTT	482	1420	597	61	67
ATAA	209	444	385	71	94
ATAA	213	502	337	69	73
ATAA	243	1110	690	85	84
ATAA	262	217	406	83	96
ATAA	342	284	189	91	80
ATAG	324	1191	908	23	58
ATAT	215	1217	483	117	88
ATCA	395	74	443	64	2564
ATCC	97	2904	805	178	120
ATCC	356	2867	2037	71	272
ATCC	393	4766	4566	647	945

ATCG	286	166	213	64	40
ATCG	400	170	263	37	38
ATCG	435	70	93	56	50
ATCT	338	260	177	33	24
ATCT	364	153	336	21	28
ATCT	367	153	218	27	23
ATGA	275	2455	1920	476	303
ATGA	453	223	117	70	61
ATGC	355	227	432	72	102
ATGC	364	290	494	92	29
ATGC	389	801	1394	104	102
ATGG	384	50	1018	72	822
ATGT	100	1369	202	49	45
ATGT	347	158	3369	3001	5830
ATGT	485	132	139	29	29
ATTA	264	1910	1632	197	91
CAAG	452	111	249	35	20
CAAT	345	1450	1473	277	338
CAAT	348	1974	1991	331	404
CACC	234	760	1892	33	115
CACC	386	240	172	87	30
CACT	189	1833	542	418	253
CACT	207	2662	985	733	446
CACT	329	27	130	722	2171
CAGA	233	1158	449	2006	629
CAGA	258	1967	448	932	199
CAGA	276	3703	1014	1287	678
CAGC	267	4437	1126	6180	1678
CAGC	359	15	282	566	909
CAGC	378	917	3020	270	1094
CAGG	421	1280	2947	262	1495
CAGG	497	106	613	5594	1325
CAGT	350	814	1068	224	432
CAGT	454	715	2136	44	59
CATA	263	1729	1492	202	114
CATA	299	763	465	92	186
CATC	142	1360	Data Not Determined	843	118
CATC	250	1264	Data Not Determined	188	273
CATC	265	3019	Data Not Determined	2761	1072
CATC	386	1843	Data Not Determined	3590	1325
CATC	452	149	Data Not Determined	144	699
CATG	351	4080	1754	180	107
CATG	354	3727	2135	252	98
CATT	76	2462	1010	207	89
CATT	268	2031	4170	807	1700
CATT	348	4125	2260	673	305
CCAA	162	1603	802	4204	283
CCAA	210	551	487	2019	716
CCAA	288	50	187	41	536
CCAA	303	576	76	7062	554
CCAC	156	2172	930	2904	1029
CCAC	292	250	173	11	8

CCAC	368	310	235	27	27
CCAG	286	259	1512	216	1942
CCAG	299	972	2058	734	335
CCAG	363	201	216	54	70
CCAG	424	256	411	73	58
CCAG	496	216	216	40	36
CCAT	176	2999	880	964	149
CCAT	328	318	174	40	30
CCCA	278	421	738	104	45
CCCA	361	4590	3881	784	490
CCCA	364	4510	3702	678	467
CCCC	123	2618	237	1694	493
CCCC	200	4439	908	3326	1944
CCCC	233	560	1187	18	17
CCCC	246	1138	438	742	156
CCCC	256	230	355	46	434
CCCC	308	910	2351	84	336
CCCC	309	910	2315	63	336
CCCC	418	38	45	324	259
CCCC	450	91	832	74	567
CCCG	140	858	213	3311	2437
CCCT	207	2136	832	188	217
CCCT	214	1223	503	159	157
CCCT	235	378	738	558	1090
CCCT	276	1538	302	14	11
CCGA	76	57	37	221	74
CCGC	149	6342	3143	2892	1950
CCGC	293	188	152	177	174
CCGC	360	85	198	803	1018
CCGG	158	3363	1432	6740	4165
CCGG	265	27	12	1982	321
CCGG	294	2442	1581	426	518
CCGT	105	2285	766	2010	1043
CCGT	130	904	331	2810	1549
CCGT	164	2586	1099	1520	386
CCGT	189	6672	4112	4680	1321
CCGT	253	3796	1758	6260	4717
CCGT	313	379	52	513	304
CCGT	351	363	219	26	11
CCGT	366	45	860	3310	5730
CCTA	142	1860	647	3232	1244
CCTA	261	3308	1467	1011	547
CCTA	396	55	162	6449	4413
CCTC	298	361	195	49	126
CCTG	171	523	204	1770	859
CCTG	184	1012	1214	285	442
CCTT	105	484	376	1235	1835
CCTT	175	2757	1279	6438	3337
CCTT	218	2713	916	341	155
CCTT	221	1242	352	672	246
CCTT	412	928	322	61	33
CGAA	193	5176	1865	1676	1583
CGAA	261	1027	2377	521	2601
CGAA	315	631	3012	2709	1099
CGAA	415	397	5018	3107	1592

CGAC	166	701	2158	698	764
CGAC	183	1477	605	1295	502
CGAC	338	183	785	880	621
CGAC	374	188	154	1142	615
CGAG	139	1266	450	2206	110
CGAG	150	4593	1800	636	159
CGAG	449	316	819	296	318
CGAT	150	2611	739	756	259
CGCA	167	501	388	2983	2353
CGCA	364	6113	4425	567	422
CGCA	472	283	150	28	36
CGCC	138	552	452	397	105
CGCC	164	569	536	55	31
CGCC	184	45	85	5730	1281
CGCG	157	6095	1869	517	521
CGCG	201	5906	4562	151	1187
CGCG	222	3906	184	3193	23
CGCG	282	129	134	2524	867
CGCG	301	124	278	57	6
CGCG	317	763	890	141	222
CGCG	491	1021	979	244	184
CGCT	168	3911	2782	381	198
CGCT	329	22	38	321	1261
CGGA	154	4444	1838	3440	1885
CGGA	183	2036	1135	2730	410
CGGA	272	2875	4547	992	1049
CGGA	305	239	2038	314	2015
CGGC	267	4605	558	5406	1067
CGGC	296	237	372	63	68
CGGC	334	201	223	2867	102
CGGG	193	1455	196	1392	151
CGGG	246	2645	1235	80	92
CGGG	324	841	297	1443	754
CGGT	164	3590	2549	3625	567
CGGT	351	1150	1618	201	262
CGGT	454	291	1511	18	12
CGTA	128	809	173	1630	586
CGTA	263	5384	3139	1163	799
CGTA	394	36	23	997	2805
CGTC	144	375	149	521	148
CGTC	165	1146	1523	3019	5618
CGTC	184	692	576	1473	385
CGTC	386	257	147	902	113
CGTG	160	1850	2216	406	427
CGTG	188	3190	1195	1024	967
CGTG	233	565	203	1324	105
CGTG	244	19	402	88	460
CGTG	268	838	2390	536	1396
CGTG	466	282	319	26	21
CGTT	186	882	509	97	79
CTAA	307	211	288	60	85
CTAA	399	340	186	30	31
CTAA	461	61	284	1540	622
CTAC	360	917	Data Not Determined	1106	2119

CTAG	366	349	1240	52	338
CTAG	372	273	1230	362	462
CTAG	406	1992	644	147	411
CTAT	132	1232	742	898	388
CTAT	132	1232	742	898	388
CTAT	321	17	32	617	242
CTAT	331	603	521	77	55
CTCA	160	1244	173	857	294
CTCA	254	1161	119	12	24
CTCA	303	563	2009	741	2477
CTCA	310	359	1478	419	1911
CTCA	363	139	134	22	45
CTCC	129	829	158	1617	305
CTCC	234	1763	2653	111	458
CTCC	392	155	431	32	21
CTCG	204	1040	234	177	102
CTCG	215	1040	91	423	47
CTCG	225	1006	77	476	51
CTCG	239	1299	424	564	340
CTCG	316	1361	2405	4700	6646
CTCG	368	24	21	60	38
CTCT	189	5873	2986	3884	1806
CTGA	272	689	364	3014	2420
CTGA	370	335	243	78	65
CTGC	118	3230	516	1373	531
CTGC	233	313	416	86	24
CTGC	336	2682	1055	47	181
CTGC	361	62	907	5745	5440
CTGC	374	32	204	2256	3407
CTGC	392	29	21	1262	863
CTGT	350	380	403	55	54
CTGT	361	93	327	1389	1465
CTTA	77	197	273	32	42
CTTC	113	994	347	160	19
CTTC	286	484	323	57	59
CTTC	311	2126	5380	229	671
CTTC	400	382	366	32	28
CTTC	470	658	338	49	47
CTTC	474	473	291	41	27
CTTC	484	1040	246	42	107
CTTG	264	1460	Data Not Determined	307	296
CTTG	276	2150	Data Not Determined	191	111
CTTG	342	322	Data Not Determined	1088	982
CTTT	286	1410	389	1397	899
CTTT	363	441	175	241	43
GAAA	180	147	263	1476	971
GAAA	317	3391	2443	1111	704
GAAA	381	24	201	643	581
GAAC	258	1069	494	1119	590
GAAC	357	741	355	436	204
GAAG	363	476	175	63	53
GAAG	406	4591	659	3156	233
GAAG	460	269	1709	2838	6960

GAAG	470	57	1034	578	2393
GAAT	159	562	604	191	149
GAAT	183	1050	183	385	182
GACA	235	416	241	41	33
GACA	250	1588	506	1532	620
GACA	271	1303	674	449	163
GACA	453	2081	954	176	43
GACC	172	1297	3369	318	472
GACC	305	940	990	239	287
GACG	262	241	427	5760	5891
GAGA	98	956	2177	97	81
GAGA	182	1045	3099	6800	6249
GAGC	155	4418	1245	1506	Data Not Determined
GAGC	359	5129	3897	822	Data Not Determined
GAGC	411	357	177	34	Data Not Determined
GAGG	111	896	3152	880	4467
GAGG	154	2048	938	1587	882
GAGG	332	155	245	49	83
GAGG	395	419	379	84	108
GAGT	366	308	634	93	62
GAGT	489	25	12	279	819
GATA	181	42	241	3348	1161
GATA	396	691	727	75	59
GATC	251	571	747	35	Data Not Determined
GATC	252	699	815	61	Data Not Determined
GATG	183	960	172	201	128
GATG	478	268	1683	104	65
GATT	153	300	716	64	299
GCAA	453	267	239	73	83
GCAC	301	3989	2016	759	429
GCAG	209	4652	985	922	316
GCAG	384	162	233	458	1652
GCAT	284	41	116	3453	4030
GCCA	162	1250	Data Not Determined	44	35
GCCA	291	64	Data Not Determined	1516	299
GCCA	459	318	Data Not Determined	1372	1049
GCCC	301	222	782	112	12
GCCG	269	2314	1775	37	64
GCCT	257	534	Data Not Determined	726	2401
GCCT	285	928	Data Not Determined	2441	52
GCCT	315	757	Data Not Determined	1183	298
GCGA	144	3325	44	472	489
GCGA	254	119	60	1290	1188
GCGA	338	1188	40	33	47
GCGA	377	95	34	454	1165
GCGA	394	14	33	703	856
GCGC	301	860	434	93	46
GCGT	262	3870	1088	1096	220

GCGT	284	26	279	6509	6353
GCTC	143	4388	2038	243	48
GCTC	326	24	716	6720	2966
GCTC	343	90	472	2610	1505
GCTC	426	5500	3372	295	497
GCTG	355	258	215	32	67
GGAG	258	640	253	1685	723
GGAG	441	1137	2278	373	479
GGAT	146	1360	362	56	67
GGCA	235	368	212	34	37
GGCA	249	2080	1002	2480	1376
GGCA	350	292	133	82	67
GGCA	410	277	1127	4299	2069
GGCA	454	3690	2362	512	254
GGCC	226	430	385	2007	2028
GGCC	255	2398	965	156	122
GGCC	430	215	355	1548	1463
GGCG	228	107	319	5775	5292
GGCG	263	188	140	851	1069
GGCG	308	18	18	29	1482
GGCT	445	887	1034	60	26
GGGA	97	404	954	102	90
GGGA	112	1786	472	156	155
GGGA	209	2657	3274	915	423
GGGA	273	252	352	1905	1353
GGGA	320	713	359	49	88
GGGA	376	51	45	316	487
GGGA	392	33	58	353	345
GGGC	88	1471	649	78	102
GGGC	493	289	212	38	15
GGGG	149	475	789	32	18
GGGT	261	2191	371	204	119
GGGT	337	161	1210	102	103
GGGT	430	444	869	355	127
GGGT	443	859	990	236	122
GGGT	465	1214	1170	272	194
GGTA	159	1100	318	102	257
GGTA	270	2005	240	37	114
GGTC	239	701	77	1570	161
GGTC	367	40	11	354	526
GGTG	87	4703	1777	490	95
GGTG	122	4627	1025	5140	2468
GGTG	189	2151	650	6550	544
GGTG	242	44	839	149	985
GGTG	256	1540	475	1330	344
GGTG	323	1427	903	753	490
GGTG	343	2363	1853	512	1870
GGTT	445	501	321	86	44
GTAA	125	1528	Data Not Determined	148	209
GTAA	190	851	Data Not Determined	5310	887
GTAA	227	509	Data Not Determined	29	23
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GTAC	300	4170	Data Not Determined	198	97
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GTAT	348	209	325	82	59
GTCA	387	55	49	1008	386
GTCA	412	64	160	286	399
GTCA	453	1106	845	98	118
GTCC	152	4663	1605	5808	2950
GTCC	188	1230	325	428	156
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GTCC	270	132	644	58	1232
GTCC	387	100	419	273	1019
GTCG	161	63	2208	96	1214
GTCT	117	4539	1148	2493	619
GTCT	211	5436	1732	275	423
GTCT	361	278	442	473	1845
GTCT	441	247	895	175	272
GTGA	146	2887	256	314	289
GTGA	209	2234	507	182	326
GTGC	402	108	610	26	23
GTGG	257	2350	2636	333	198
GTGG	265	2198	2134	339	282
GTGG	322	421	1269	834	653
GTGT	285	407	797	4191	5084
GTTA	266	3333	3390	358	278
GTTA	323	320	619	63	55
GTTC	220	1380	218	921	167
GTTC	426	2590	1650	153	185
GTTG	123	3838	659	2292	743
GTTG	244	45	1180	6242	6175
TAAA	94	2302	552	126	159
TAAA	320	515	177	84	52
TAAC	395	1326	611	106	115
TAAG	214	2811	371	60	64
TAAG	387	603	367	100	98
TAAT	188	1420	95	129	69
TAAT	238	95	731	775	825
TACA	238	1292	401	395	155
TACC	470	325	244	41	25
TACG	94	1378	669	1630	721
TACG	409	1490	777	451	268
TACT	323	1599	1405	157	246
TACT	358	278	220	77	127
TACT	375	214	124	1564	1135
TAGC	99	628	401	52	110
TAGC	267	94	157	2297	2826
TAGC	288	512	1394	239	366
TAGC	467	124	132	47	44
TAGG	82	927	478	139	35

TAGG	126	2866	261	102	27
TAGG	264	62	95	3750	769
TAGG	295	329	208	45	21
TAGG	307	958	341	549	248
TAGT	99	85	75	1183	498
TAGT	164	996	323	93	36
TAGT	349	115	237	57	41
TAGT	452	180	208	44	44
TATA	249	32	113	1753	1610
TATA	362	22	21	85	2693
TATA	386	121	336	74	35
TATA	411	1940	2420	387	613
TATC	288	622	79	2156	53
TATC	452	675	211	109	58
TATG	266	30	265	4030	1407
TATG	375	1886	459	285	267
TATG	410	2313	303	131	132
TCAA	146	1390	58	1252	119
TCAA	228	288	1369	265	2468
TCAA	249	6650	1250	667	97
TCAA	481	37	429	215	1224
TCAC	211	851	915	61	79
TCAC	277	1832	1263	109	189
TCAG	149	3936	3296	639	740
TCAG	212	1639	715	247	146
TCAG	263	11	478	2908	4625
TCAT	216	85	352	62	81
TCAT	338	103	375	2202	4086
TCAT	374	323	244	49	21
TCAT	410	16	53	2281	2070
TCCC	282	1400	608	1004	534
TCCC	371	552	241	1218	359
TCCT	141	99	269	1482	1270
TCCT	310	501	355	28	35
TCGC	123	1612	2788	1060	1498
TCGG	112	1666	1447	88	255
TCGG	314	1055	696	53	32
TCGG	417	204	362	56	52
TCGT	167	4690	1973	343	311
TCGT	396	290	207	74	35
TCGT	461	750	391	99	108
TCTA	314	389	93	1051	40
TCTG	93	1988	437	302	147
TCTG	264	84	124	4400	1057
TCTT	296	231	545	5923	2696
TCTT	317	20	39	1187	1049
TGAC	302	3410	1412	837	751
TGAC	327	1490	1208	147	50
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TGAG	154	586	246	107	98
TGAG	399	25	411	1581	1964
TGCA	387	209	132	26	21
TGCC	268	488	729	111	87
TGCC	360	743	68	2852	101
TGCT	193	716	336	94	95

TGCT	315	525	291	33	56
TGGG	292	1024	586	21	38
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TGTA	291	187	548	59	54
TGTA	303	481	306	52	42
TGTA	331	3156	2046	240	432
TGTA	411	640	712	88	75
TGTC	183	1299	353	33	35
TGTC	368	234	180	28	50
TGTG	289	82	477	54	554
TGTG	411	4850	1200	784	70
TGTT	404	85	248	57	359
TTAC	289	31	2124	2940	6362
TTAG	137	447	456	40	36
TTAT	188	5220	1491	1424	415
TTCA	390	301	291	82	61
TTCA	468	495	314	88	88
TTCC	315	770	795	116	83
TTCG	211	1370	1242	212	338
TTCG	218	2510	1585	223	353
TTCG	265	50	77	730	195
TTCG	308	171	223	96	497
TTCG	409	286	190	65	51
TTCT	189	1012	342	2037	286
TTGC	288	255	998	115	255
TTGC	347	117	94	39	35
TTGG	262	474	515	6364	3238
TTGG	465	190	435	66	62
TTGT	467	102	175	54	32
TTTA	249	27	38	238	200
TTTA	265	1097	924	75	51
TTTC	122	205	145	4154	1987
TTTC	348	110	248	51	73
TTTC	437	773	463	81	78
TTTG	265	30	716	5214	2891
TTTG	397	358	165	41	50
TTTT	327	1166	226	51	55

TABLE 2

Seq ID	Clone ID	Digital Address (Msp1)	Gene Match (Accession #)	% Homology	DST Bases matched/total DST bases	Nucleotide homology	
						DST nucleotide range (bp#)	Database nucleotide range (bp#)
18	MM_3	AAGT 366	M. glucocorticoid-attenuated response gene 49 (U43086)	100%	311/311	1 – 311	1396 - 1706
24	MM_6	ATGG 384	Mus musculus adult C57BL/6J testis Mus musculus cDNA clone (AV044899.2)	97%	308/317	14 – 330	1 - 317
23	MM_7	ATCA 395	R. H-rev107 mRNA (X76453)	87%	186/212	58 – 269	417 - 628
1	MM_11	AGGT 315	Novel (strong EST hit) (AA543723)	98%	144/146	1 - 146	363 - 508
2	MM_12	ACAA 381	380 MMU25096 [H] Mus musculus Kruppel-like factor LKLF mRNA (U25096)	99%	228/230	96 – 325	1308 - 1537
3	MM_13	ACGT 347	m. Cytosolic aspartate aminotransferase isoenzyme (J02623)	98%	270/273	1 - 273	1676- 1949
4	MM_14	TATA 249	M. mRNA for RIP1 gene (X80937)	97%	110/113	69 – 181	3531 - 3643
5	MM_15	TAGC 267	Mus musculus PYS-2 mRNA (M23901)	99%	207/209	1 – 209	238 - 246
6	MM_16	TTTA 265	Mouse mitochondrial genome (V00711)	98%	204/207	1 – 207	8401 - 8607
7	MM_17	TTTG 265	M. Y-box binding protein 1/DNA binding protein B mRNA (M60419)	98%	184/186	20 – 205	1136 - 1321
8	MM_18	TTGG 262	M. mRNA for transcript overlapping myelin basic protein gene (X67319)	100%	79/79	61 – 139	4508 - 4586
9	MM_19	TGAG 399	M. MHC class I H-2 classical transplantation antigen mRNA (M131797)	97%	318/325	1 – 325	102 - 427
10	MM_20	TGTG 411	Glutathione peroxidase (MMGSHPX or X03920)	99%	356/358	1 – 358	970 - 1329
11	MM_21	TCAT 410	Novel (strong EST hit) (AA 183527)	99%	358/360	1 - 360	112 - 471
12	MM_22	TCTT 296	Novel (strong EST hit) (AA 122524)	98%	235/238	7 - 244	4 - 241

TABLE 2 (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Gene Match (Accession #)	%Homology	DST Bases matched/total DST bases	Nucleotide homology	
13	MM_23	TCGG 314	DDP-like protein (AF165967); (AF15087); (C80966)	99% (C80966)	254/256	1 - 256	2 - 257
14	MM_26	GTTG 244	Novel (strong EST hit) (AA271535)	98%	190/191	1 - 191	219 - 409
15	MM_27	GTTC 426	R.G protein gamma-5 subunit (M95780)	94%	209/221	1 – 221	88 - 308
16	MM_28	GGCT 445	444 MMNDIPKB [M] Mus musculus mRNA for nucleoside diphosphate kinase B. (X68193)	98%	392/396	1 – 396	204 - 600
17	MM_29	GCAT 284	Mouse B1 repeat 2.7 kb downstream from c-mos oncogene (X01141)	89%	106/119	41 – 159	56 - 174
19	MM_30	GCGT 284	Mouse B1 repeat 2.7 kb downstream from c-mos oncogene (X01141)	88%	105/118	41 – 158	56 - 173
20	MM_32	CATA 263	Mouse Mitochondrial genome (V00711)	99%	205/207	1 – 207	8401 - 8607
25	MM_37	CGGG 246	Mouse surfeit locus surfeit 3 gene, exon 8, and surfeit 1 and 2 (M14689)	99%	123/124	69 – 192	232 - 355
21	MM_38	CGGC 267	Mus musculus mRNA regulated by bone marrow morphogenetic protein (X95281)	97%	201/207	1 – 207	652 - 858
22	MM_40	CCCC 450	R. endoplasmic reticulum alpha-mannosidase mRNA (M57547)	91%	277/303	17 – 319	2983 - 3285

TABLE 3: VERIFIED CANDIDATE MATCHES

Seq ID	Clone ID	Digital Address (Msp1)	Gene Identity (Accession #)	Extended Primer
18	MM_3	AAGT 366	M. glucocorticoid-attenuated response gene 49 (U43086)	GAT CGA ATC CGG AAG TGT GTC AGA GTG CAG
24	MM_6	ATGG 384	Mus musculus adult C57BL/6J testis Mus musculus cDNA clone (AV044899.2)	GAT CGA ATC CGG ATG GCA ACC AGA TGA TTG
23	MM_7	ATCA 395	R. H-rev107 mRNA (X76453)	GAT CGA ATC CGG ATC ATC CAG CGG GCT GAG
	MM_9	AGTC 323	323 MUSPCT [M] Mus musculus p6-5 gene, 3' end (M27347)	GAT CGA ATC CGG AGT CCA GTG GCC TCC CCA
1	MM_11	AGGT 315	Novel (strong EST hit) (AA543723)	GAT CGA ATC CGG AGG TAC GTG AGA GAA TTC
2	MM_12	ACAA 381	380 MMU25096 [H] Mus musculus Kruppel-like factor LKLF mRNA (U25096)	GAT CGA ATC CGG ACA AGT GTG GCC ACA GGA
3	MM_13	ACGT 347	M. Cytosolic aspartate aminotransferase isoenzyme (J02623)	GAT CGA ATC CGG ACG TGA CTG TGG GTG TTG
4	MM_14	TATA 249	M. mRNA for RIP1 gene (X80937)	GAT CGA ATC CGG TAT ACA ACA TCC ACT TTA
5	MM_15	TAGC 267	Mus musculus PYS-2 mRNA (M23901)	GAT CGA ATC CGG TAG CCT GTC AGC TCC ATG
6	MM_16	TTTA 265	Mouse mitochondrial genome (V00711)	GAT CGA ATC CGG TTT ACA GCT AAC ATT ACT
7	MM_17	TTTG 265	M. Y-box binding protein 1/DNA binding protein B mRNA (M60419)	GAT CGA ATC CGG TTT GGT CAT CCA ACA GGG
8	MM_18	TTGG 262	M. mRNA for transcript overlapping myelin basic protein gene (X67319)	GAT CGA ATC CGG TTG GCA CAG CCA TCA ACT
9	MM_19	TGAG 399	M. MHC class 1 H-2 classical transplantation antigen mRNA (M131797)	GAT CGA ATC CGG TGA GCC TAT GGA CTC AAT
10	MM_20	TGTG 411	Glutathione peroxidase (MMGSHPX) or (X03920)	GAT CGA ATC CGG TGT GCC GCA ACG ACA TTG
11	MM_21	TCAT 410	Novel (strong EST hit) (AA 183527)	GAT CGA ATC CGG TCA TGT ATT GTA TCC ATG

TABLE 3: VERIFIED CANDIDATE MATCHES (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Gene Identity (Accession #)	Extended Primer
12	MM_22	TCTT 296	Novel (strong EST hit) (AA 122524)	GAT CGA ATC CGG TCT TAA CAG AGG ACT CCT
13	MM_23	TCGG 314	DDP-like protein (AF165967); (AF15087); (C80966)	GAT CGA ATC CGG TCG GTT TGC CCA GAT CGT
14	MM_26	GTTG 244	Novel (strong EST hit) (AA271535)	GAT CGA ATC CGG GTT GCA CCT ATT GCA TGT
15	MM_27	GTTC 426	R.G protein gamma-5 subunit (M95780)	GAT CGA ATC CGG GTT CAA CCG CGT GAA GGT
16	MM_28	GGCT 445	444 MMNDIPKB [M] Mus musculus mRNA for nucleoside diphosphate kinase B. (X68193)	GAT CGA ATC CGG GGC TGG TGA AGT ACA TGA
17	MM_29	GCAT 284	Mouse B1 repeat 2.7 kb downstream from c-mos oncogene (X01141)	GAT CGA ATC CGG GCA TGG TGG CGC ACG GGT
19	MM_30	GCGT 284	Mouse B1 repeat 2.7 kb downstream from c-mos oncogene (X01141)	GAT CGA ATC CGG GCG TGG TGG CGC ACG GGG
20	MM_32	CATA 263	Mouse Mitochondrial genome (V00711)	GAT CGA ATC CGG CAT ACA GCT AAC ATT ACT
25	MM_37	CGGG 246	Mouse surfeit locus surfeit 3 gene, exon 8, and surfeit 1 and 2 (M14689)	GAT CGA ATC CGG CGG GCC CAT CGG AGG ACA
21	MM_38	CGGC 267	Mus musculus mRNA regulated by bone marrow morphogenetic protein (X95281)	GAT CGA ATC CGG CGG CCA CCC AAC AAC TTT
22	MM_40	CCCC 450	R. endoplasmic reticulum alpha-mannosidase mRNA (M57547)	GAT CGA ATC CGG CCC CTG ACA CCA TCT GGA

SEQUENCE LISTING

<110> Carson, Monica J
Sutcliffe, J. Gregor
Almazan, Melissa T.
Tobal, Gabriela M.

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atttctgagt ttgaggccag cctggtctac agagtgagtt cgaggacaac cagggctaca 120
cagagaaacc ctgtctcgaa aaccaaaaac aaaaaaaaaa gaactccagt taagacttct 180
aataccaaat tctcttgcaa gttatgaaaa taaagtatat aaamcgaaaa a 231

<210> 18
<211> 317
<212> DNA
<213> Mus musculus

<400> 18
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gattgctgac aaactgctct tgattgtttc tttaaggaac tgctttctct ccctgactcc 180
tctgctcatc ctagccatac aattttccag tcagcaaacc tcattactaa tcatgtaggg 240
aatggagctt aaagcagaca gagcaccttt tgatcacatt ttttttctct gcaataaatg 300
acaactccca acaaaaa 317

<210> 19
<211> 232
<212> DNA
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atttctgagt ttgaggccag cctggtctac agagtgagtt cgaggacaac cagggctaca 120
cagagaaacc ctgtctcgag aaccaaaaac aaaaaaaaag aactccagtt aagacttcta 180
ataccaaatt ctcttgcaag ttatgaaaat aaagtatata aaaggacaaa aa 232

<210> 20
<211> 211
<212> DNA
<213> Mus musculus

<400> 20
ccggcataca gctaacatta ctgcaggaca cttattaata cacctaatcg gaggagctac 60
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actcacaatt ctagaatttg cagtagcatt aattcaagcc tacgtattca ccctcctagt 180
aagcctatat ctacatgata atacacaaaa a 211

<210> 21
<211> 216
<212> DNA
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<400> 21
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actctgcctt tccctccaaa accctctcac tcccagctcg tgcaaactgg ttacacagca 180
gaaacgcaaa ataaagaggt ggctttcgca taaaaa 216

<210> 22
<211> 380
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ggtngcatct ttgtnctgta 380

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<213> Mus musculus

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<210> 24
<211> 335
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ctgtaataaa ataattcgat gactatctgg aaaaa 335

<210> 25
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cctacctggt acggactgtg tgcggccaca tcatatttgt ggttccaaaa atttgtacgt 120
cggacaccca tcatgtgaag taaggagcct gttcccaaca accataaaat aaagatcttg 180
ctatgcaaaa a 191

<210> 26
<211> 49
<212> DNA
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<223> Description of Artificial Sequence: cDNA anchor primer

<400> 26
gaattcaact ggaagcggcc cgcaggaatt tttttttttt ttttttvnn 49

<210> 27
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' RT primer

<400> 27
aggtcgacgg tatcgg 16

<210> 28
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer

<400> 28
ggtcgacggt atcggn 16

<210> 29
<211> 15
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: universal 3' PCR primer

<400> 29
gagctccacc gcggt 15

<210> 30
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer

<400> 30
cgacggtatc ggnnnn 16

<210> 31
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases G-T-T-C

<400> 31
cgacggtatc gggttc 16

<210> 32
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence:5' PCR primer with parsing bases G-T-T-G

<400> 32
cgacggtatc gggttg 16

<210> 33
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases A-A-G-T

<400> 33
cgacggtatc ggaagt 16

<210> 34
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases A-G-G-T

<400> 34
cgacggtatc ggaggt 16

<210> 35
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases A-C-A-A

<400> 35
cgacggtatc ggacaa 16

<210> 36
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-A-T-A

<400> 36
cgacggtatc ggtata 16

<210> 37
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-T-G-G

<400> 37
cgacggtatc ggttgg 16

<210> 38
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-G-T-G

<400> 38
cgacggtatc ggtgtg 16

<210> 39
<211> 16

<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-C-A-T

<400> 39
cgacggtatc ggtcat 16

<210> 40
<211> 16
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-C-G-G

<400> 40
cgacggtatc ggtcgg 16

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_11

<400> 41
gatcgaatcc ggaggtacgt gagagaattc 30

<210> 42
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_12

<400> 42
gatcgaatcc ggacaagtgt ggccacagga 30

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_13

<400> 43
gatcgaatcc ggacgtgact gtgggtgttg 30

<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_14

<400> 44
gatcgaatcc ggtatacaac atccacttta 30

<210> 45
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_15

<400> 45
gatcgaatcc ggtagcctgt cagctccatg 30

<210> 46
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_16

<400> 46
gatcgaatcc ggtttacagc taacattact 30

<210> 47
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_17

<400> 47
gatcgaatcc ggtttggtca tccaacaggg 30

<210> 48
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_18

<400> 48
gatcgaatcc ggttggcaca gccatcaact 30

<210> 49
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer
for clone MM_19

<400> 49
gatcgaatcc ggtgagccta tggactcaat 30

<210> 50
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_20

<400> 50
gatcgaatcc ggtgtgccgc aacgacattg 30

<210> 51
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_21

<400> 51
gatcgaatcc ggtcatgtat tgtatccatg 30

<210> 52
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_22

<400> 52
gatcgaatcc ggtcttaaca gaggactcct 30

<210> 53
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_23

<400> 53
gatcgaatcc ggtcggtttg cccagatcgt 30

<210> 54
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_26

<400> 54
gatcgaatcc gggttgcacc tattgcatgt 30

<210> 55
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_27

<400> 55
gatcgaatcc gggttcaacc gcgtgaaggt 30

<210> 56
<211> 30
<212> DNA
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<223> Description of Artificial Sequence: extended TOGA primer for MM_28

<400> 56
gatcgaatcc ggggctggtg aagtacatga 30

<210> 57
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_29

<400> 57
gatcgaatcc gggcatggtg gcgcacgggt 30

<210> 58
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_3

<400> 58
gatcgaatcc ggaagtgtgt cagagtgcag 30

<210> 59
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_30

<400> 59
gatcgaatcc gggcgtggtg gcgcacgggg 30

<210> 60
<211> 30

<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_32

<400> 60
gatcgaatcc ggcatacagc taacattact 30

<210> 61
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_38

<400> 61
gatcgaatcc ggcggccacc caacaacttt 30

<210> 62
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_40

<400> 62
gatcgaatcc ggcccctgac accatctgga 30

<210> 63
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_7

<400> 63
gatcgaatcc ggatcatcca gcgggctgag 30

<210> 64
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for clone MM_6

<400> 64
gatcgaatcc ggatggcaac cagatgattg 30

<210> 65
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for MM_37

<400> 65
gatcgaatcc ggcgggccca tcggaggaca 30

<210> 66
<211> 30
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: extended TOGA primer for MM_9

<400> 66
gatcgaatcc ggagtccagt ggcctcccca 30

<210> 67
<211> 252
<212> DNA
<213> Mus musculus

<400> 67
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cagaaggcgc aattcactgc gcaggtgcat cacttcatgg aactatgttg ggataagtgt 120
gtggagaagc caggaagtcg gctagactcc cgcactgaaa actgcctctc tagctgtgtg 180
gatcgcttca ttgacactac tcttgccatc accggtcggt ttgcccagat cgtacagaaa 240
ggagggcagt ag 252

<210> 68
<211> 24
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: cloning primer for MM_23

<400> 68
atggccgagc ttggtgaagc ggac 24

<210> 69
<211> 24
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: cloning primer for MM_23

<400> 69
ctgccctcct ttctgtacga tctg 24

We claim:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:1-25 or a polynucleotide which is hybridizable to SEQ ID NO:1-25;

(b) a polynucleotide encoding a polypeptide fragment of a translation of SEQ ID NO: 1-25 or a polypeptide fragment encoded by the cDNA sequence which is hybridizable to SEQ ID NO:1-25;

(c) a polynucleotide encoding a polypeptide epitope of a translation of SEQ ID NO: 1-25 or a polypeptide epitope encoded by a cDNA sequence which is hybridizable to SEQ ID NO:1-25;

(e) a polynucleotide encoding a polypeptide of a translation of SEQ ID NO: 1-25, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:1-25;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:1-25;

(h) a polynucleotide which encodes a species homologue of a translation of SEQ ID NO: 1-25;

(i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the polypeptide sequence identified as a translation of SEQ ID NO: 1-25 or the polypeptide encoded by the cDNA which is hybridizable to SEQ ID NO:1-25.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:1-25 or the cDNA sequence which is hybridizable to SEQ ID NO:1-25.

5 . The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6 . The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of a translation of SEQ ID NO: 1-25;
(b) a polypeptide domain of a translation of SEQ ID NO: 1-25;
(c) a polypeptide epitope of a translation of SEQ ID NO: 1-25;
(d) a secreted form of a translation of SEQ ID NO: 1-25;
(e) a full length protein of a translation of SEQ ID NO: 1-25;
(f) a variant of a translation of SEQ ID NO: 1-25;
(g) an allelic variant of a translation of SEQ ID NO: 1-25; and
(h) a species homologue of a translation of SEQ ID NO: 1-25.

12. The isolated polypeptide of claim 11, wherein the secreted form or the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO: 1-25.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:1-25 in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 22.

24. A substantially pure isolated DNA molecule suitable for use as a probe for genes regulated in macrophages and microglia, chosen from the group consisting of the DNA molecules identified in Table 1, having a 5' partial nucleotide sequence and length as described by their digital address, and having a characteristic regulation pattern in treated and untreated microglia and macrophages.

25. A kit for detecting the presence of a polypeptide of the present invention in a mammalian tissue sample comprising an antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to the polynucleotide of the present invention or with a polypeptide encoded by the polynucleotide of the present invention in an amount sufficient for at least one assay and suitable packaging material.

26. A kit of claim 25 further comprising a detecting antibody which binds to the antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to

the polynucleotide of the present invention or with a polypeptide encoded by the polynucleotide of the present invention.

27. A kit of claim 26 wherein the detecting antibody is labeled.

28. A kit of claim 27 wherein the label comprises enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, or bioluminescent compounds.

29. A kit for detecting the presence of genes encoding an protein comprising a polynucleotide of claims 1 or 2, or fragment thereof having at least 10 contiguous bases, in an amount sufficient for at least one assay, and suitable packaging material.

30. A method for detecting the presence of a nucleic acid encoding a protein in a mammalian tissue sample, comprising the steps of:

(a) hybridizing a polynucleotide of claims 1 or 2, or fragment thereof having at least 10 contiguous bases, with the nucleic acid of the sample; and

(b) detecting the presence of the hybridization product.

31. A marker suitable for indicating an inflammatory response in the central nervous system comprising an isolated polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 48, 51, 53, 54, and 58;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 48, 51, 53, 54 and 58;

(c) a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 48, 51, 53, 54 and 58; and

(d) a polynucleotide fragment capable of hybridizing under stringent conditions to a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 48, 51, 53, 54 and 58.

32. A kit for indicating an inflammatory response in the central nervous system comprising the marker of claim 31.

33. A method of indicating an inflammatory response in the central nervous system comprising the step of contacting a sample with the marker of claim 31.

34. A marker suitable as a cell-specific marker for microglia comprising an isolated polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 41, 42, 44, 53, and 57;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 41, 42, 44, 53, and 57;

(c) a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 41, 42, 44, 53, and 57; and

(d) a polynucleotide fragment capable of hybridizing under stringent conditions to a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 41, 42, 44, 53, and 57.

35. A kit for the specific detection of microglia comprising the marker of claim 34.

36. A method of detecting microglia in a sample comprising the step of contacting a sample with the marker of claim 34.

37. A marker suitable as a cell-specific marker for activated microglia comprising an isolated polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 58;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 58;

(c) a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 58; and

(d) a polynucleotide fragment capable of hybridizing under stringent conditions to a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 58.

38. A kit for the specific detection of activated microglia comprising the marker of claim 37.

39. A method of detecting activated microglia in a sample comprising the step of contacting a sample with the marker of claim 37.

40. A marker suitable for indicating an autoimmune disease in the central nervous system comprising an isolated polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 48, 51;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 48, 51;

(c) a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 48, 51; and

(d) a polynucleotide fragment capable of hybridizing under stringent conditions to a polynucleotide fragment encoding a polypeptide fragment of a translation of SEQ ID NO: 48, 51.

41. A kit for indicating an autoimmune disease in the central nervous system comprising the marker of claim 40.

42. A method of detecting an autoimmune disease in the central nervous system comprising the step of contacting a sample with the marker of claim 40.

43. A marker suitable for indicating an inflammatory response in the central nervous system wherein the marker is an antibody specifically immunoreactive with a polypeptide fragment encoded by a polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 48, 51, 53, 54, and 58;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 48, 51, 53, 54 and 58.

44. A kit for indicating an inflammatory response in the central nervous system comprising the marker of claim 43.

45. A method of detecting an inflammatory response in the central nervous system comprising the step of contacting a sample with the marker of claim 43.

46. A marker suitable as a cell-specific marker for microglia wherein the marker is an antibody specifically immunoreactive with a polypeptide fragment encoded by a polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 48, 51, 53, 54, and 58;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 48, 51, 53, 54 and 58.

47. A kit for the specific detection of microglia comprising the marker of claim 46.

48. A method of detecting microglia in a sample comprising the step of contacting a sample with the marker of claim 46.

49. A marker suitable as a cell-specific marker for activated microglia wherein the marker is an antibody specifically immunoreactive with a polypeptide fragment encoded by a polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 58;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 58;

50. A kit for the specific detection of activated microglia comprising the marker of claim 49.

51. A method of detecting activated microglia in a sample comprising the step of contacting a sample with the marker of claim 49.

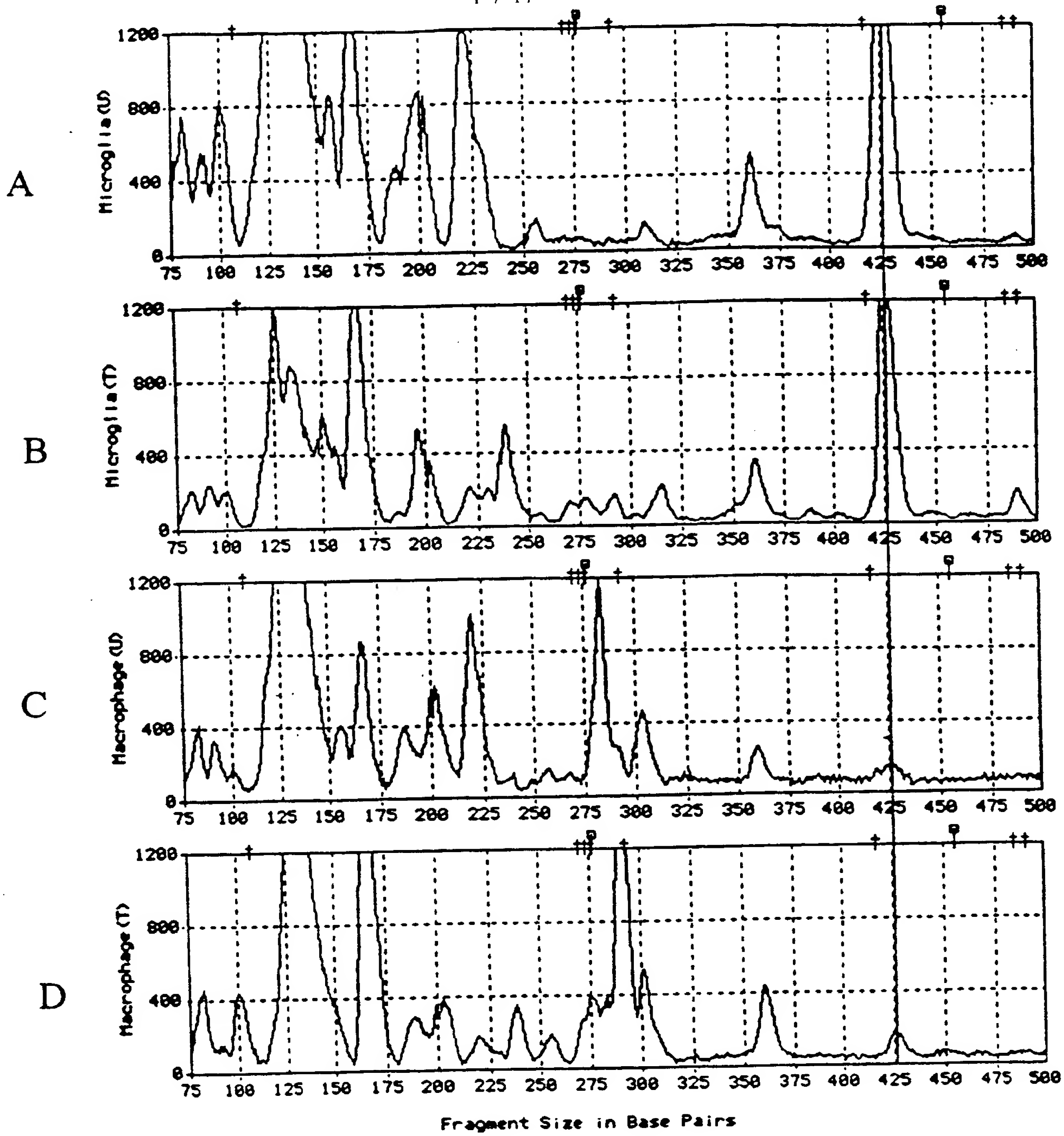
52. A marker suitable for indicating an autoimmune disease wherein the marker is an antibody specifically immunoreactive with a polypeptide fragment encoded by a polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO: 48, 51;

(b) a polynucleotide that is capable of hybridizing under stringent conditions to SEQ ID NO: 48, 51.

53. A kit for indicating an autoimmune disease in the central nervous system comprising the marker of claim 52.

54. A method of detecting an autoimmune disease in the central nervous system comprising the step of contacting a sample with the marker of claim 52.



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Intercepts: 2590 1650 153 185

Figure 1

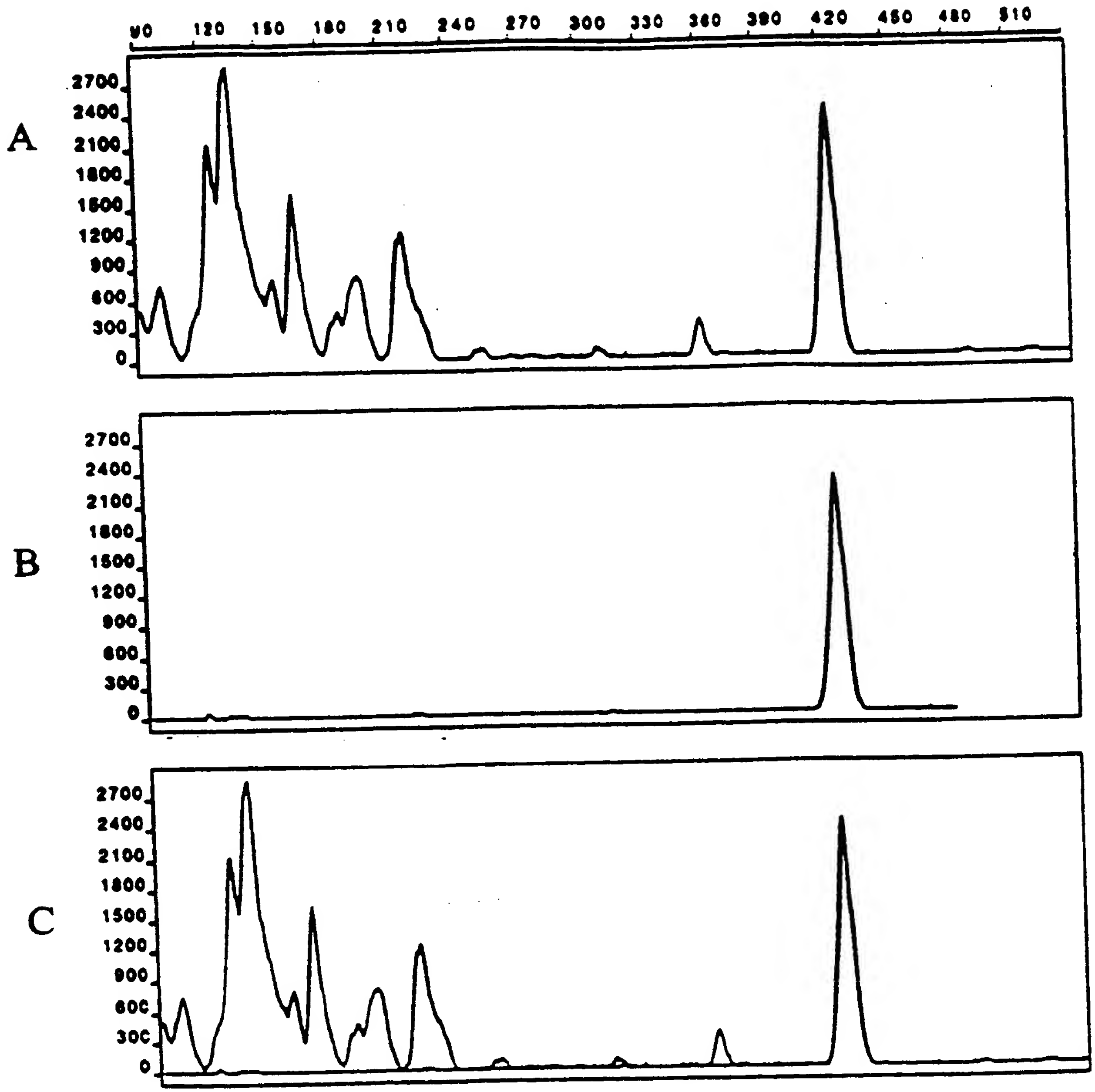
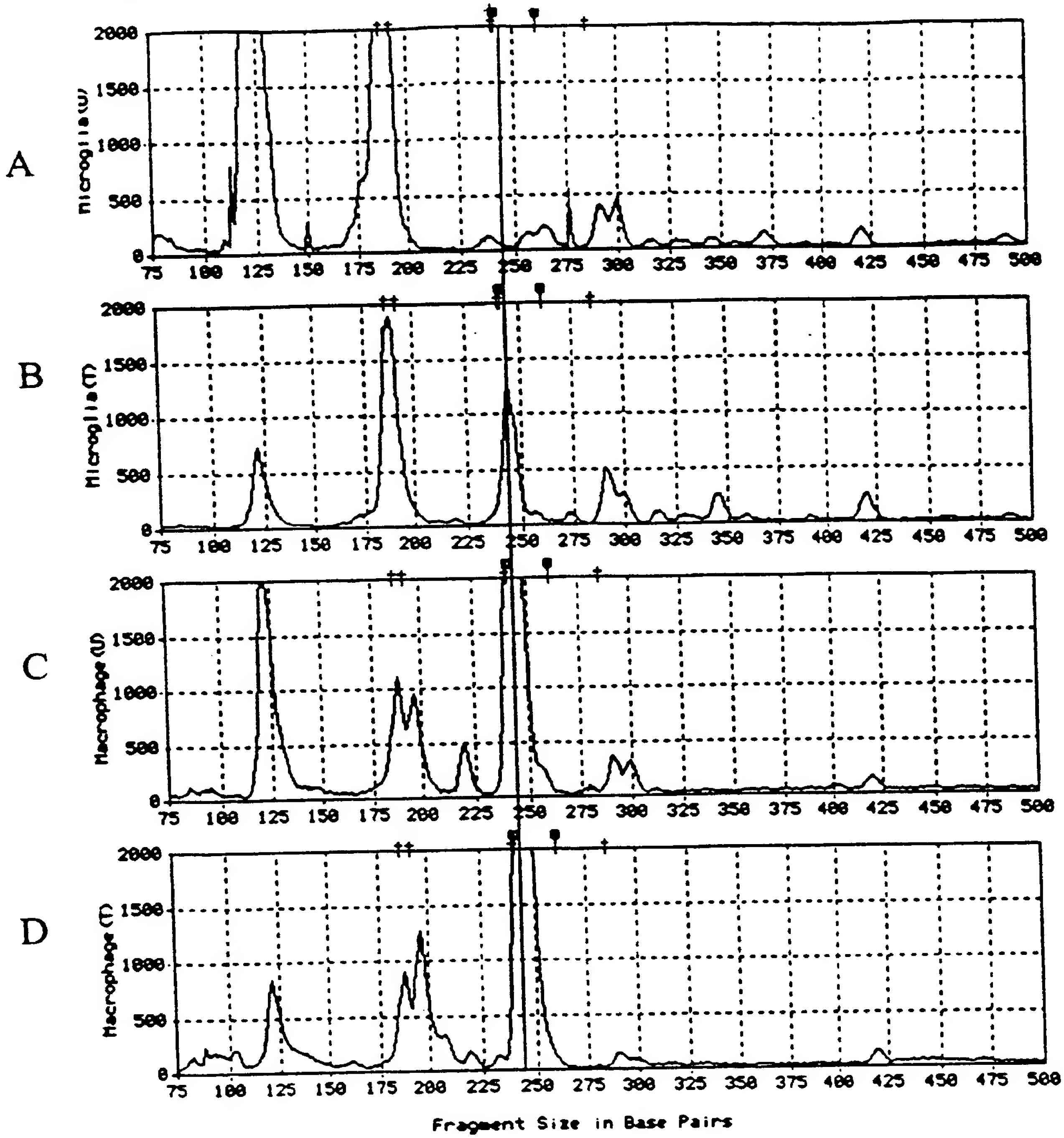

90
120
150
180
210
240
270
300
330
360
390
420
450
480
510
A
B
C
2700
2400
2100
1800
1500
1200
900
600
300
0

Figure 2



GTTG : 244, on Scale Mt0_2000
Intercepts: 56 1230 6700 6185

Figure 3

A

B

C

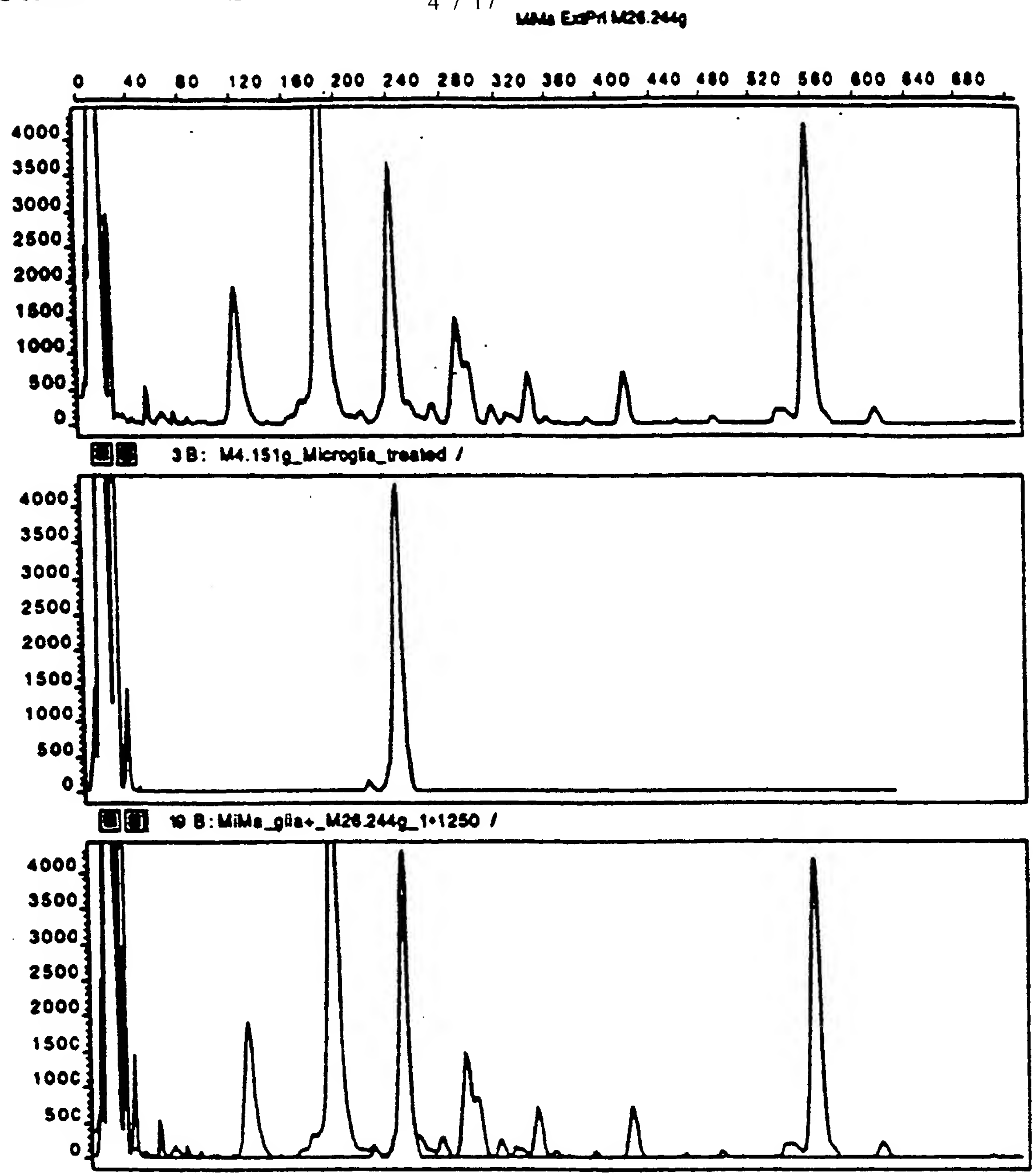

MiMa ExtPri M26.244g
0 40 80 120 160 200 240 280 320 360 400 440 480 520 560 600 640 680
4000
3500
3000
2500
2000
1500
1000
500
0
3 B: M4.151g_Microglia_treated /
19 B: MiMa_glia+_M26.244g_1+1250 /

Figure 4

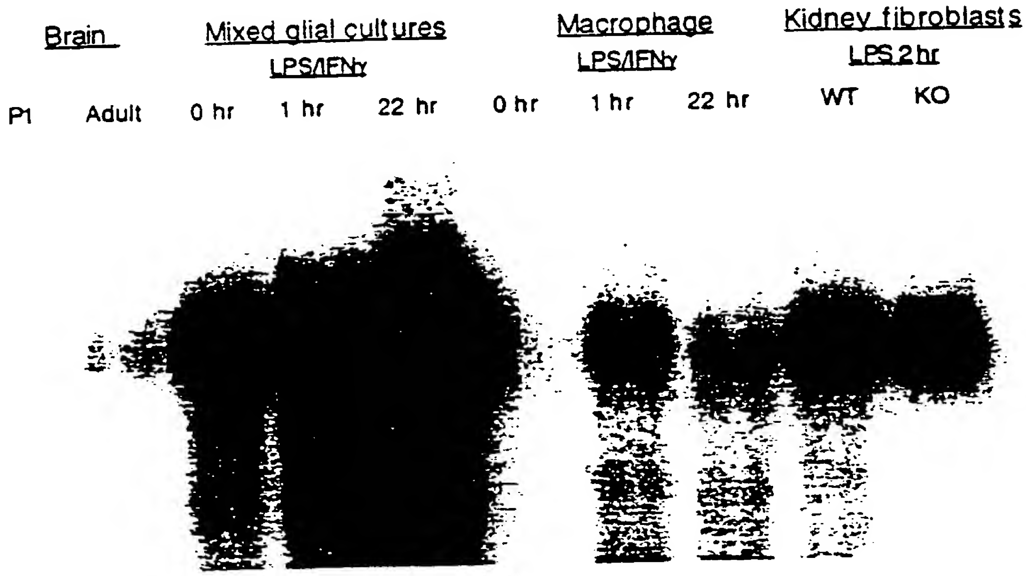


Figure 5A

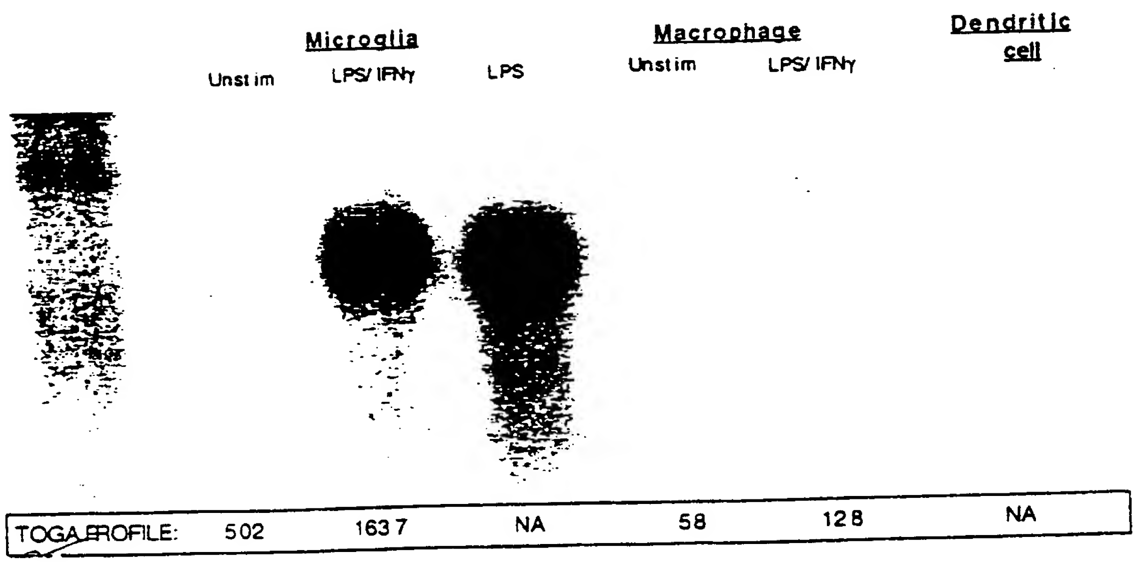


Figure 5B

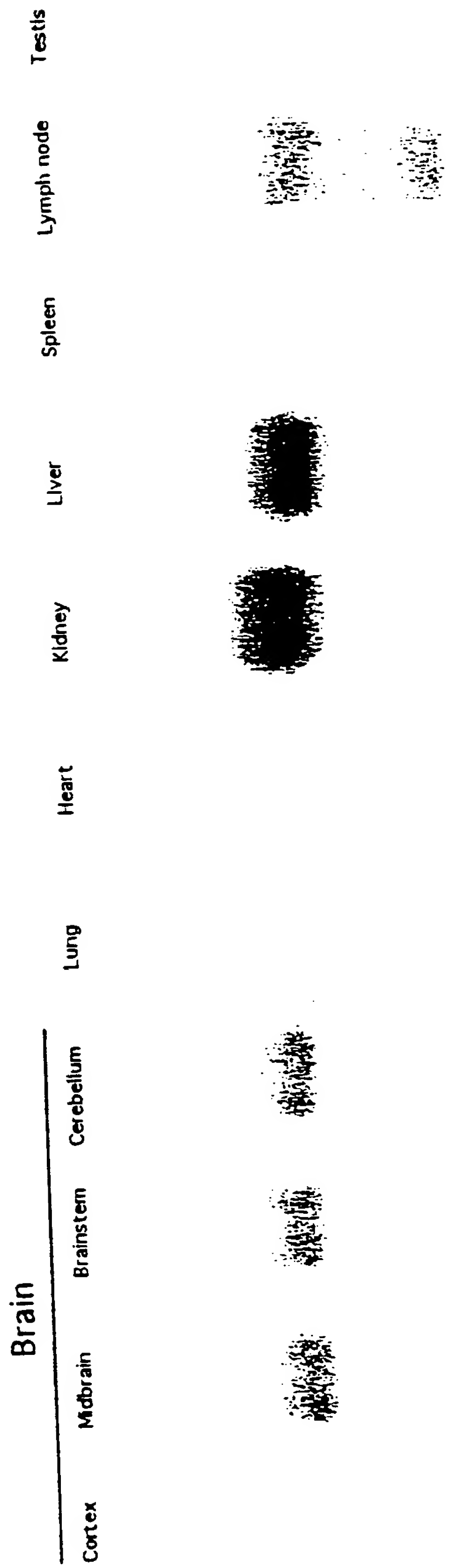

Brain
Cortex
Midbrain
Brainstem
Cerebellum
Lung
Heart
Kidney
Liver
Spleen
Lymph node
Testis

Figure 5C

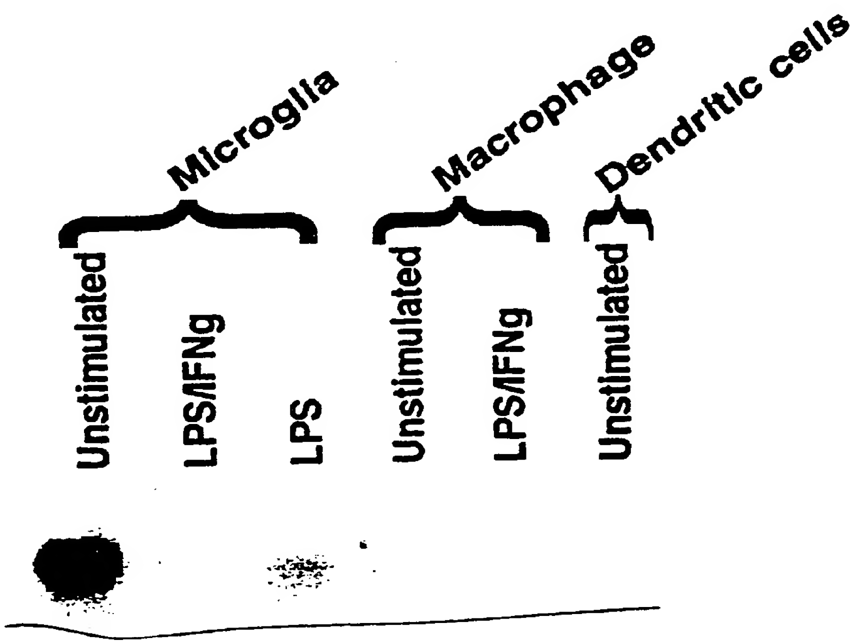

Microglia
Macrophage
Dendritic cells
Unstimulated
LPS/IFNg
LPS
Unstimulated
LPS/IFNg
Unstimulated

Figure 6

Figure 7A

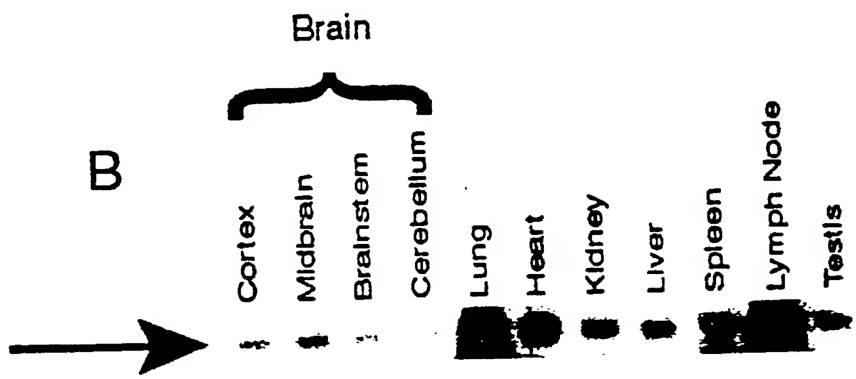


Figure 7B

A.

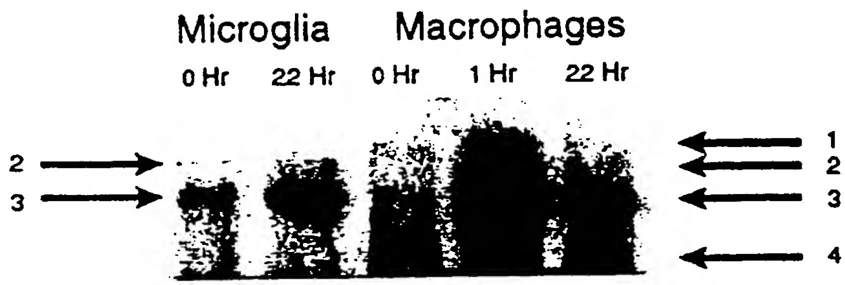


Figure 8A

B.

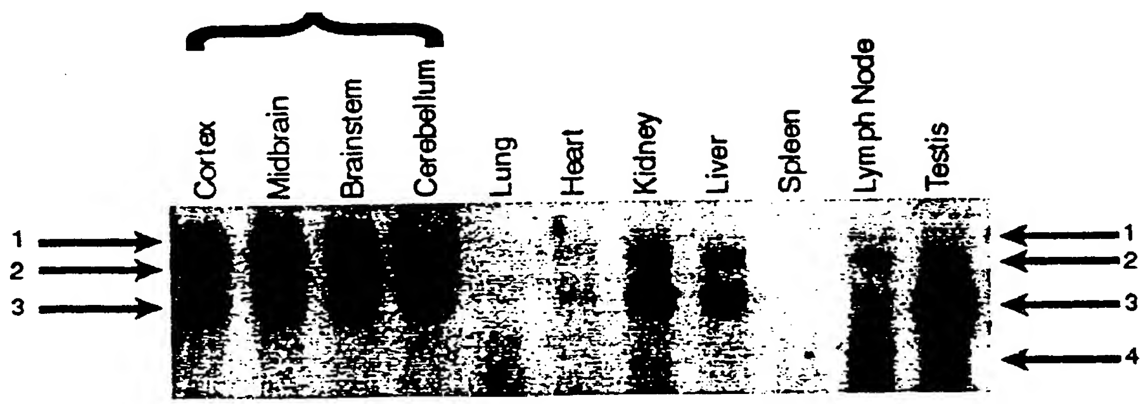
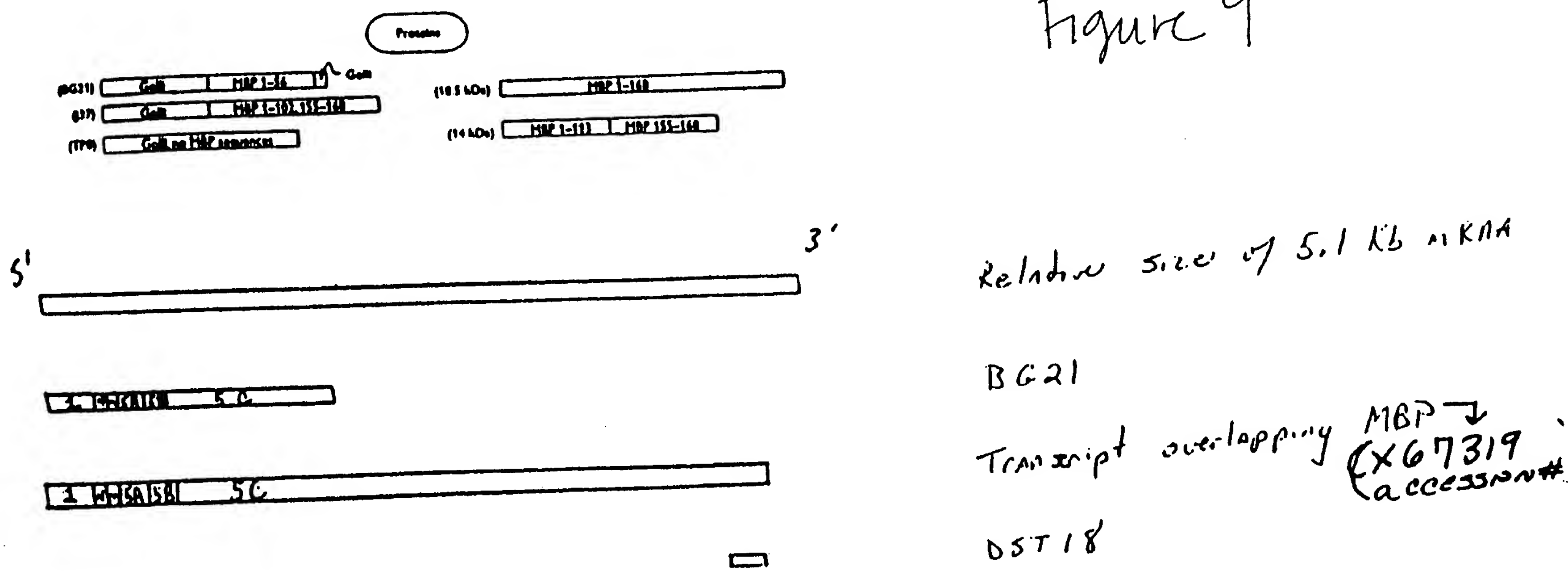


Figure 8B

Figure 9


Proteins
(BG21) Golli MBP 1-56 Golli
(J37) Golli MBP 1-102,155-168
(TP8) Golli no MBP sequences
(18.5 kDa) MBP 1-168
(14 kDa) MBP 1-113 MBP 155-168
5'
3'
Relative size of 5.1 Kb mRNA
BG21
Transcript overlapping MBP
X67319
accession #
DST18

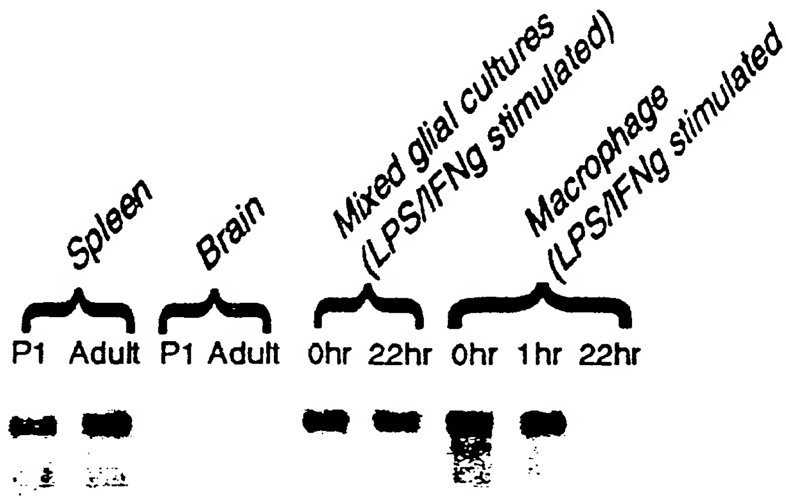

Spleen
Brain
Mixed glial cultures
(LPS/IFNg stimulated)
Macrophage
(LPS/IFNg stimulated
P1
Adult
P1
Adult
0hr
22hr
0hr
1hr
22hr

Figure 10

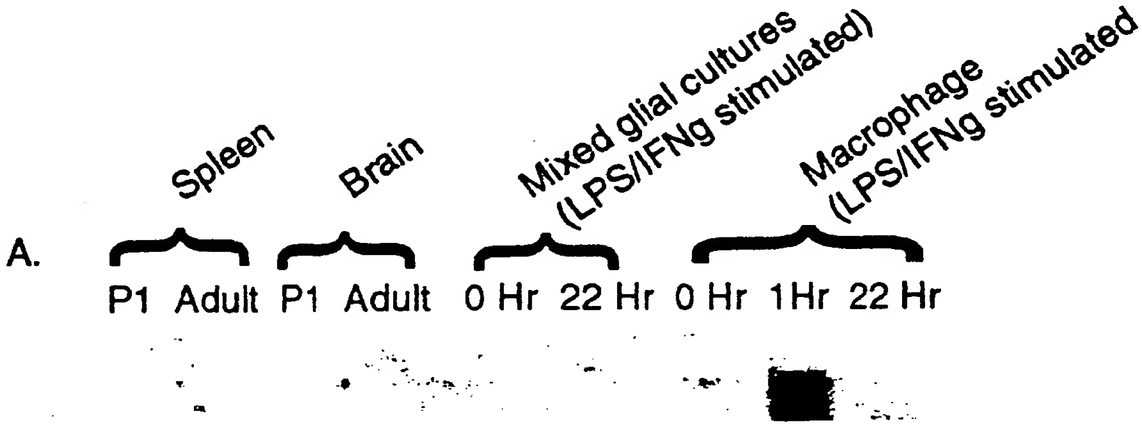


Figure 11A

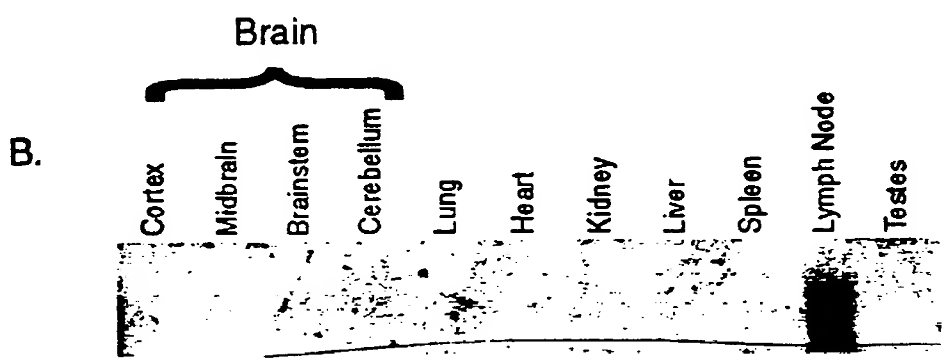


Figure 11B

A.

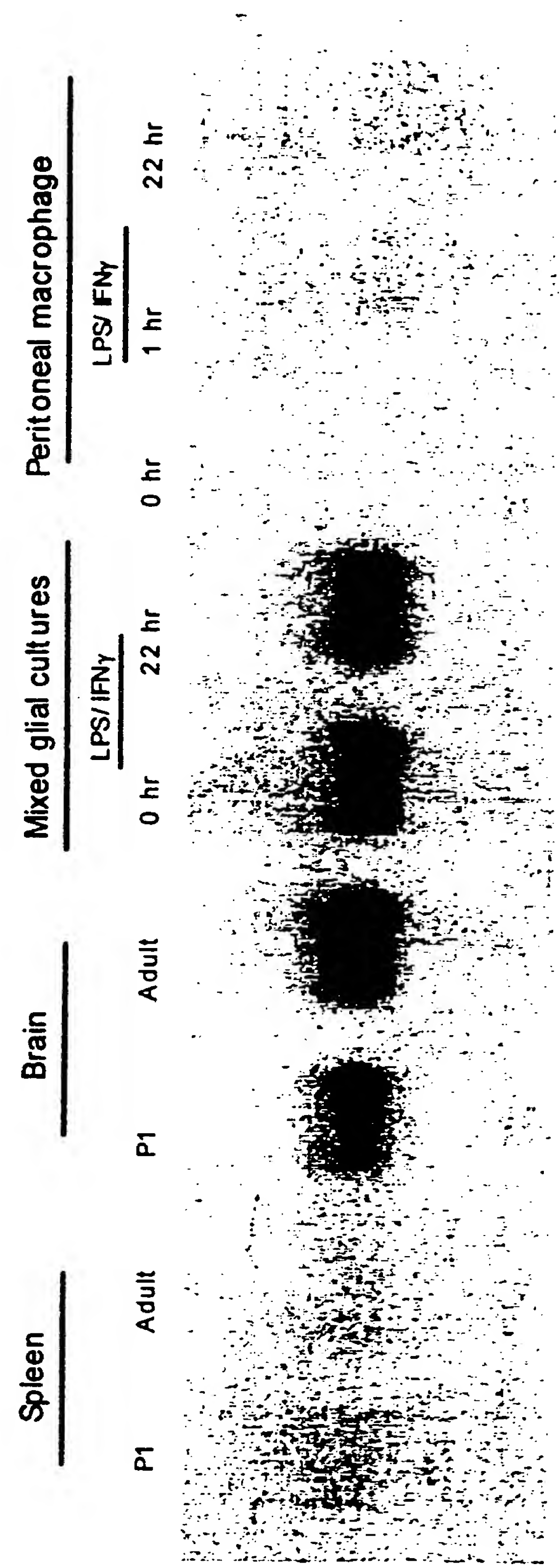


Figure 12A

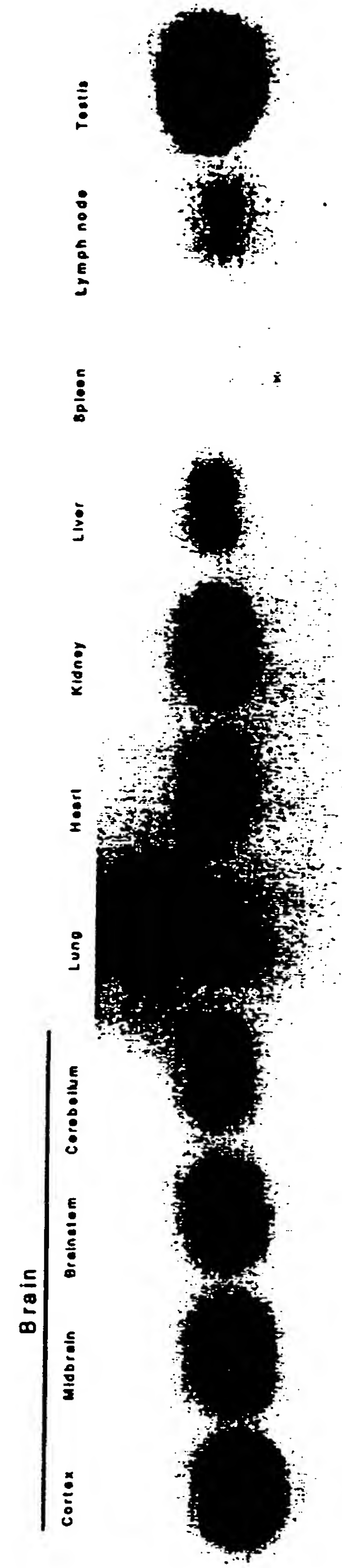
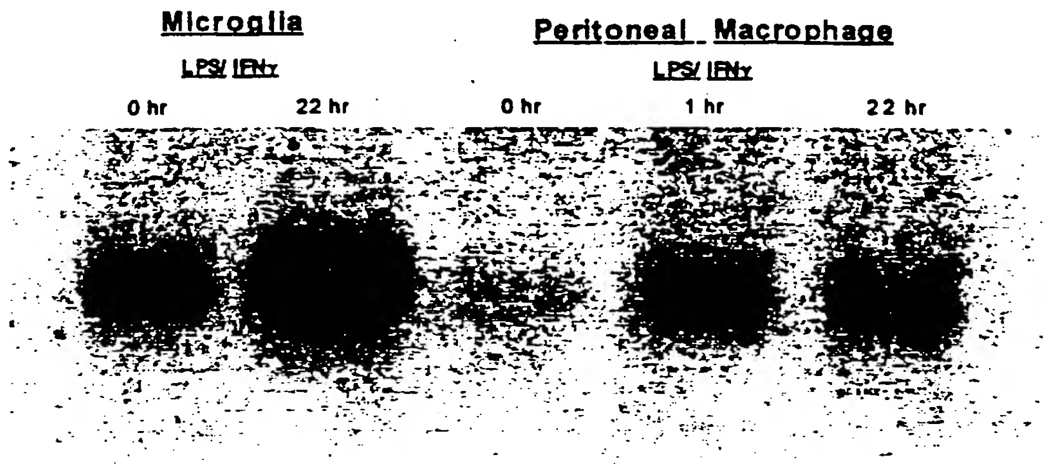


Figure 12B



TOGA Profile: 1055	696	53	NA	32

Figure 12C

human	DDP	MDSSSSSSAA	GLGAVDP.QL	QHFIEVETQK	QRFQQLVHQM	TELCWEKCMD	49
yeast	SPAC13G6.04	MADATKNPIA	DLSESEQLEL	SKFIESEQQK	VKLQQATHQF	TSTCWPKCIG	50
human	.yv59a08.s1	....	....	PGWPPSOPEG	RSLXAQVHHF	MELCWDKCVE	
mouse	.DST23	MA	ELGEADEAEL	QRLVAAEQQK	AQFTAQVHHF	MELCWDKCVE	
	DDP	KPGPKLDSRA	EACFVNCVER	FIDTSQFILN	RLEQT.QKSK	PVFSESL.SD*	97
	SPAC13G6.04	NIGNKLDKSE	EQCLQNCVER	FLDCNFHIIK	RYALEKFGFL	..FCWLGFSC*	98
	.yv59a08.s1	KPGNRLDSRT	ENCLSSCVDR	FIDTTLAITS	RFAQIVQKGG	Q*	
	.DST23	KPESRLDSRT	ENCLSSCVDR	FIDTTLAITG	RFAQIVQKGG	Q*	

Figure 13

PCR Primers

```
      5'ORF

    ATGGCCGAGCTTGGTGAAGCGGACGAAGCGGAGTTACAACGCCTGGTGGCCGCCGAACAG
  1 ---------+---------+---------+---------+---------+---------+ 60
    TACCGGCTCGAACCACTTCGCCTGCTTCGCCTCAATGTTGCGGACCACCGGCGGCTTGTC

    M  A  E  L  G  E  A  D  E  A  E  L  Q  R  L  V  A  A  E  Q  -

    CAGAAGGCGCAATTCACTGCGCAGGTGCATCACTTCATGGAACTATGTTGGGATAAGTGT
 61 ---------+---------+---------+---------+---------+---------+ 120
    GTCTTCCGCGTTAAGTGACGCGTCCACGTAGTGAAGTACCTTGATACAACCCTATTCACA

    Q  K  A  Q  F  T  A  Q  V  H  H  F  M  E  L  C  W  D  K  C  -

    GTGGAGAAGCCAGGAAGTCGGCTAGACTCCCGCACTGAAAACTGCCTCTCTAGCTGTGTG
121 ---------+---------+---------+---------+---------+---------+ 180
    CACCTCTTCGGTCCTTCAGCCGATCTGAGGGCGTGACTTTTGACGGAGAGATCGACACAC

    V  E  K  P  G  S  R  L  D  S  R  T  E  N  C  L  S  S  C  V  -

    GATCGCTTCATTGACACTACTCTTGCCATCACCGGTCGGTTTGCCCAGATCGTACAGAAA
181 ---------+---------+---------+---------+---------+---------+ 240
    CTAGCGAAGTAACTGTGATGAGAACGGTAGTGGCCAGCCAAACGGGTCTAGCATGTCTTT

    D  R  F  I  D  T  T  L  A  I  T  G  R  F  A  Q  I  V  Q  K  -

    GGAGGGCAGTAG                                         3'ORF
241 ---------+-- 252
    CCTCCCGTCATC

    G  G  Q  *  -
```

Figure 14

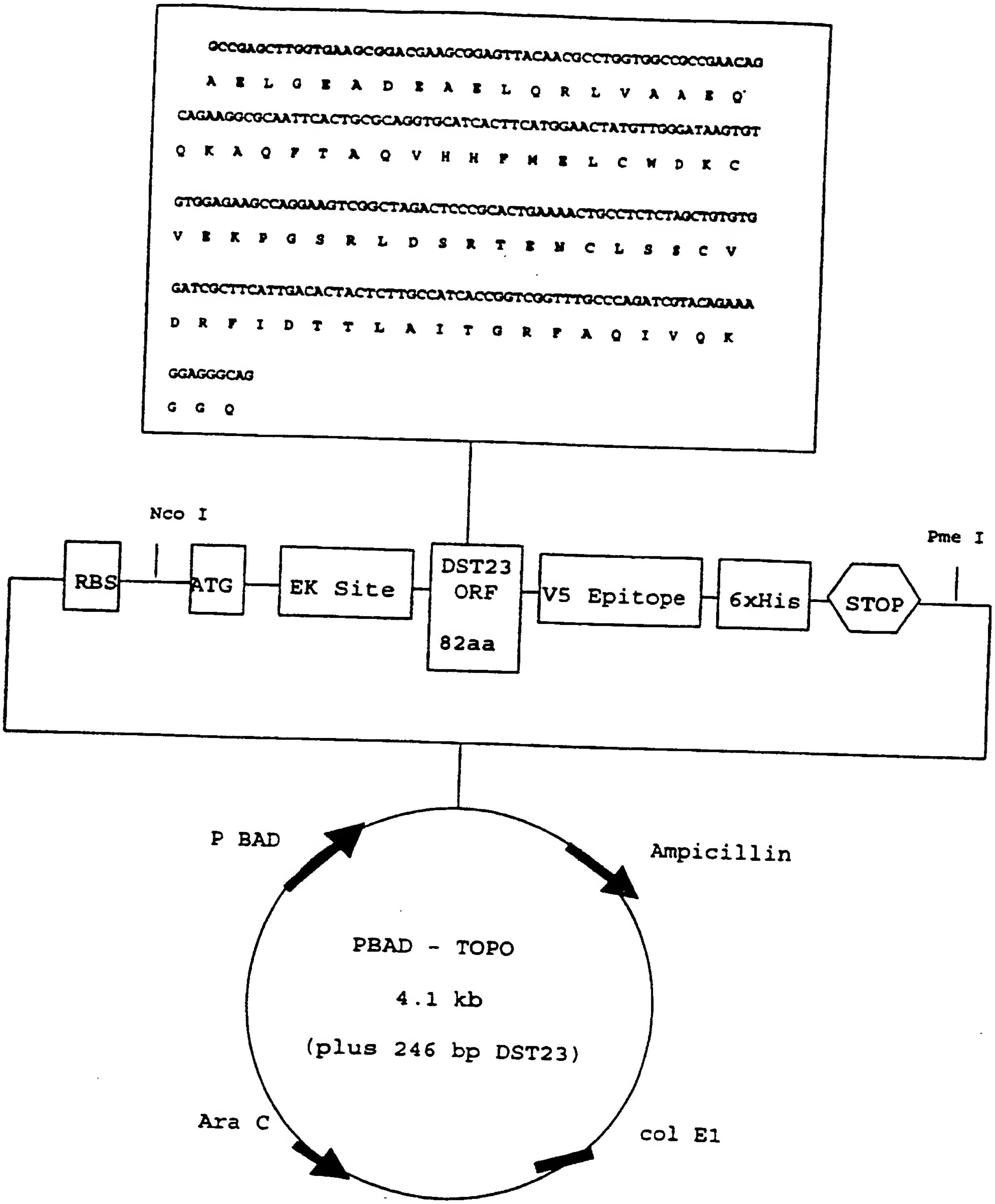

GCCGAGCTTGGTGAAGCGGACGAAGCGGAGTTACAACGCCTGGTGGCCGCCGAACAG
A E L G E A D E A E L Q R L V A A E Q
CAGAAGGCGCAATTCACTGCGCAGGTGCATCACTTCATGGAACTATGTTGGGATAAGTGT
Q K A Q F T A Q V H H F M E L C W D K C
GTGGAGAAGCCAGGAAGTCGGCTAGACTCCCGCACTGAAAACTGCCTCTCTAGCTGTGTG
V E K P G S R L D S R T E N C L S S C V
GATCGCTTCATTGACACTACTCTTGCCATCACCGGTCGGTTTGCCCAGATCGTACAGAAA
D R F I D T T L A I T G R F A Q I V Q K
GGAGGGCAG
G G Q
Nco I
RBS
ATG
EK Site
DST23 ORF 82aa
V5 Epitope
6xHis
STOP
Pme I
P BAD
Ampicillin
PBAD - TOPO
4.1 kb
(plus 246 bp DST23)
Ara C
col E1

Figure 15